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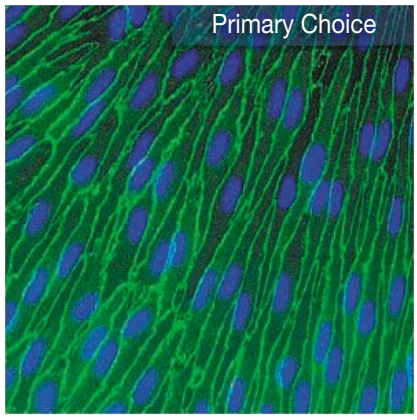

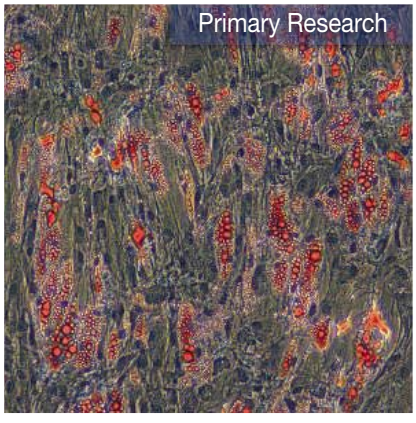

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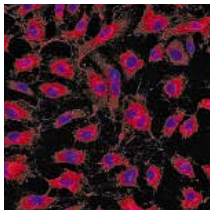

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COVER

Artist's reconstruction of *Castorocauda lutrasimilis* diving into water. *Castorocauda* is an omnivorous mammal from the Middle Jurassic (~164 million years ago) and is the earliest known semi-aquatic animal in the mammalian lineage. See page 1123.

Image: Mark A. Klingler, Carnegie Museum of Natural History

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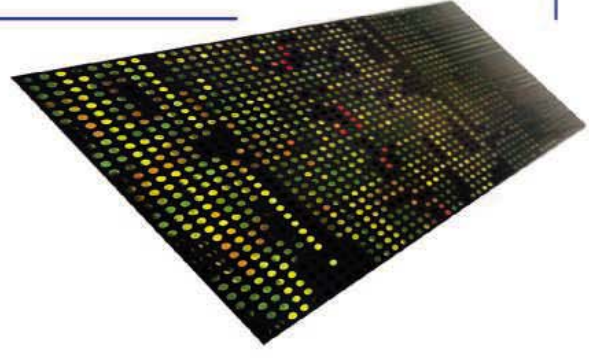

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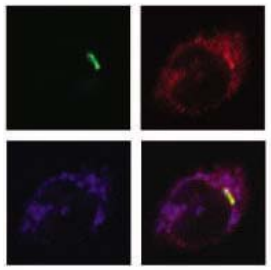

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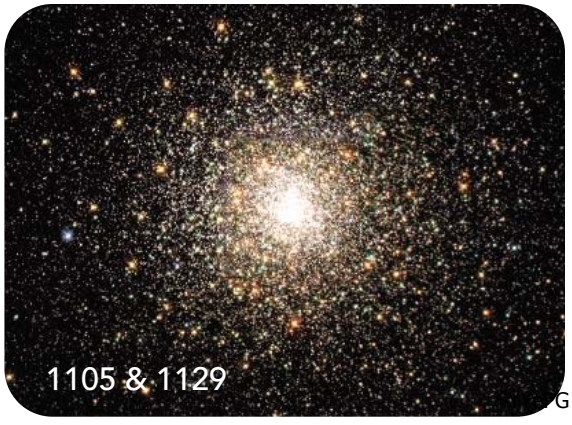

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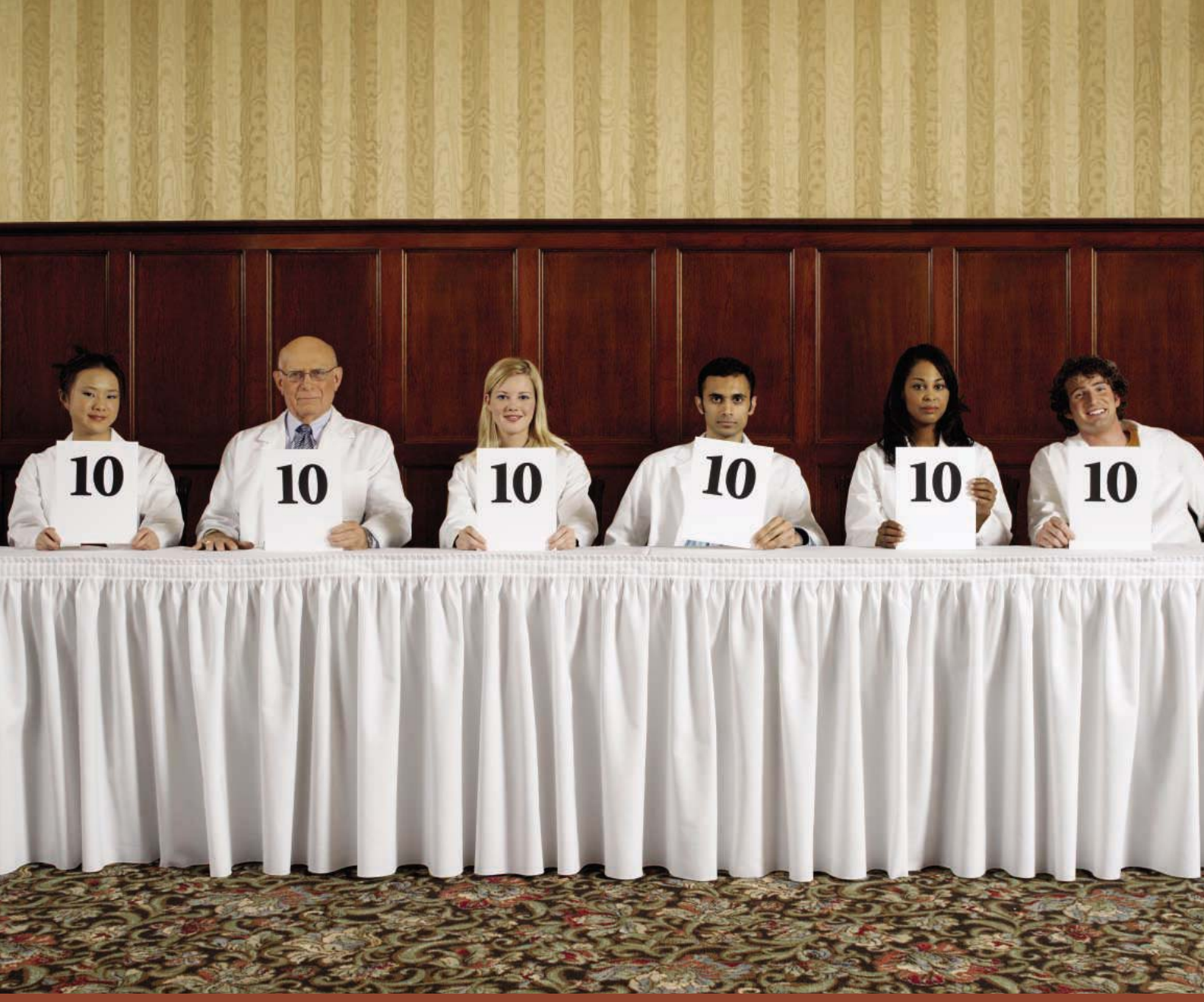

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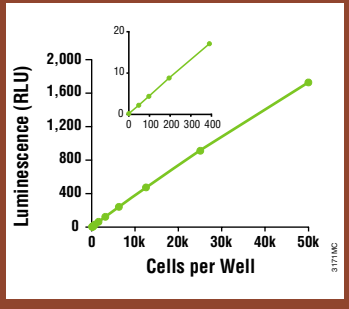

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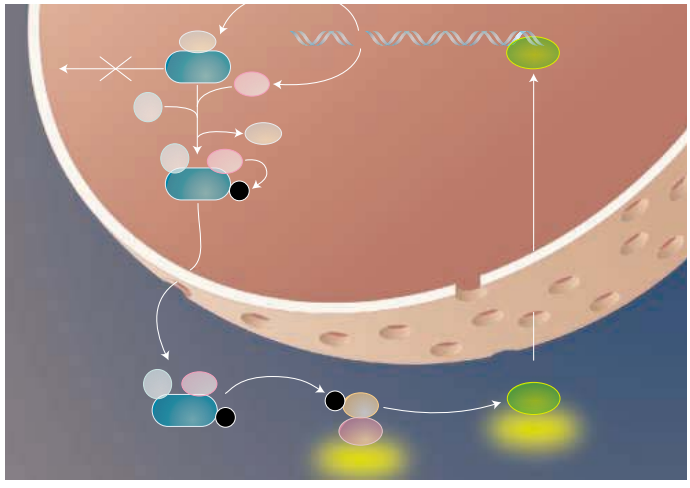

1110 & 1141

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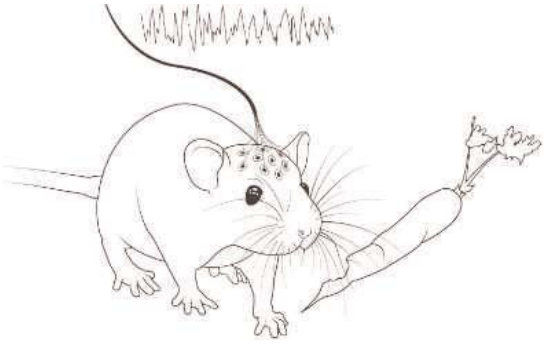

Vitamin A and cortical synchrony.

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PERSPECTIVE: Meeting Report—Barossa 2005, Signaling Networks
M. A. Guthridge, G. J. Goodall, S. M. Pitson
Highlights from this meeting show how knowledge of signaling complexity reveals insight into disease.

PERSPECTIVE: Retinoic Acid Signaling in the Functioning Brain
U. C. Dräger
The locations and mechanisms through which retinoic acid affects cortical synchrony in the mature brain remain a mystery.

SCIENCE'S SAGE KE

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NEWS FOCUS: The Sour Side of Sweet
M. Leslie
Sugar derivatives take a toll on cells.

CLASSIC PAPER: Detection of Inactive Enzyme Molecules in Ageing Organisms
H. Gershon and D. Gershon
Accumulation of nonfunctional proteins may play a key role in senescence; *Nature* **227**, 1214 (1970).

SCIENCENOW

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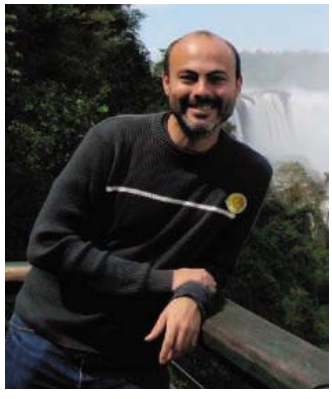
The Case of Mistaken IQ
New test indicates autistics are smarter than people think.

Turning Buildings on Their Heads
New computer program could allow architects to create more complex structures.

Spit Hides Clues to Disease
Researchers find markers for breast cancer and diabetes in human saliva.



Returning home to Argentina.

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GLOBAL: Crossing Continents
A. Forde
Molecular biologist Javier Palatnik talks about his experiences in the U.S. and Europe and why he returned to his native Argentina.

US: Guarding the Wire—Working in Computer Security
A. Fazekas
Next Wave talks with U.C. Berkeley professor and computer security expert David Wagner about succeeding in this field.

MISCINET: Educated Woman, Chapter 48—Micella Hits the Road
M. P. DeWhyse
Micella talks to other students about the trials and tribulations of graduate school.

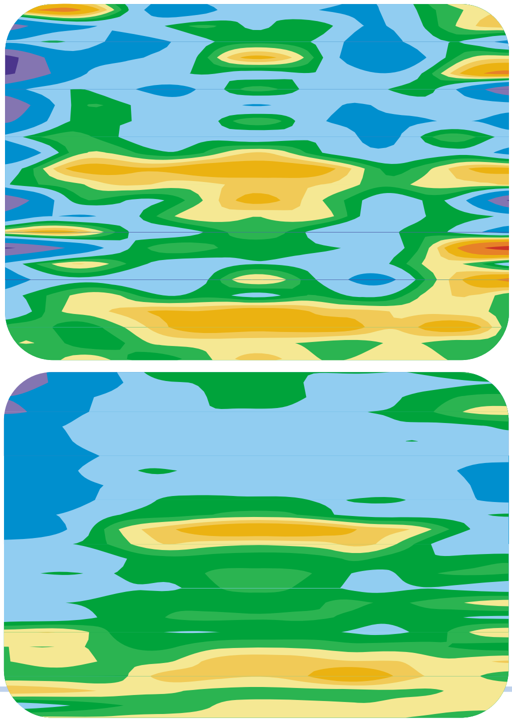
MISCINET: MentorDoctor—Suffering From a Lack of Direction
ScienceCareers Staff
The MentorDoctor team helps a Ph.D. student who feels his adviser isn't helping keep his project on track.

GRANTSNET: International Grants and Fellowship Index
A. Kotok
Learn about the latest funding opportunities from Europe, Asia, and the Americas.

EDITED BY STELLA HURTLEY AND PHIL SZUROMI

Dissecting Stratospheric Temperature Trends

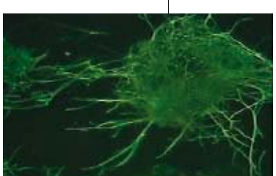
Since 1980, the lower stratosphere has cooled significantly. This cooling trend has been ascribed to the influence of anthropogenic effects—mainly stratospheric ozone depletion and the buildup of greenhouse gases. However, this process occurred in two major steps. **Ramaswamy *et al.*** (p. 1138) investigated the temporal structure of the trend using simulations with a climate model, in order to delineate the roles of natural and anthropogenic forcings. Although the overall downward trend in temperature is the result of anthropogenic factors, natural forcing by changes in solar irradiance and volcanic aerosols have superimposed on the gradual longer term decrease the shorter time-scale structure recorded in the observations. Thus, while anthropogenic factors are responsible for the 25-year-long stratospheric cooling trend, the steps were caused by natural forcing.



Early Aquatic Mammal

Mesozoic mammals have been thought to have been small, nocturnal, and confined to a few niches on land until the demise of the dinosaurs 65 million years ago. Most are recorded by isolated jaw fragments or teeth. **Ji *et al.*** (p. 1123; see the cover and the Perspective by **Martin**) now describe a Jurassic mammal from China that breaks this mold. The fossil is well preserved, and impressions of fur can be seen on its body and scales on a broad tail (similar to a beaver overall). The animal was fairly large, approaching not quite half a meter in length, and the shape of its limbs suggest that it was adapted for swimming and burrowing. The combination of both primitive and derived features in this early mammal, and the demonstration that mammals had occupied aquatic habitats by this time, expands the evolutionary innovations of early mammals.

Segregating Old and New Chromatids



During chromosome replication, paired chromatids ultimately separate during cell division to become individual chromosomes in daughter cells. Although one might expect segregation of chromatids (with old versus newly synthesized strands) to daughter cells to be random, some studies have suggested that nonrandom segregation can occur. **Armakolas and Klar** (p. 1146) looked for evidence of chromosome-specific nonrandom strand segregation in various cell types. After mitotic recombination, mouse chromosome 7 shows random segregation in cardiomyocytes, pancreatic, and mesoderm cells, whereas nonrandom segregation is seen in embryonic stem cells, endoderm cells, and neuroectoderm cells. These segregation patterns may be important for developmental decisions and have implications for imprinting and inheritance.

Wind Up

Recent evidence suggests that short-duration gamma-ray bursts are produced by fast mergers of compact objects, such as double-neutron stars and neutron-star, black-hole binaries. However, lingering x-ray emissions seen hundreds of seconds after some bright gamma-ray bursts are still a problem for this model because simulations predict the merger should happen in seconds. **Dai *et al.*** (p. 1127) suggest that the merger process proceeds less catastrophically, producing a differentially rotating millisecond pulsar rather than a final black hole. Because the pulsar's layers spin at different rates, its magnetic fields become wound up and release energy sporadically through reconnection-driven explosive x-ray flares.

Red and Blue

Globular star clusters in elliptical galaxies come in two colors, red or blue. Many astronomers have assumed the colors reflected age differences, such that blue clusters formed more recently than red ones, and implying two epochs of globular cluster formation during the growth history of elliptical galaxies. **Yoon *et al.*** (p. 1129, published online 19 January; see the Perspective by **Freeman**), however, show that a single coeval population of globular clusters can exhibit color bimodality due to a nonlinear relationship between color and metallicity in stars. Galactic spectral models that include treatment of horizontal branch stars can reproduce the color distributions even with stars of similar age, removing the need for multiple populations of globular clusters.

Finding the Path for Quantum Computing

Quantum computers hold great promises for solving difficult problems otherwise intractable on classical computers. However, actually finding algorithms, or the quantum circuitry on which the algorithms can be implemented, is challenging because the number of components in the quantum circuits should grow only polynomially with the complexity of the problem you want to solve. While manipulation of a single qubit can be thought of as the rotation of a unit vector in a sphere, a quantum computer will typically have n interacting qubits, giving rise to a 2^n-dimensional space, Thus **Nielsen *et al.*** (p. 1133; see the Perspective by **Oppenheim**) recast the problem of finding an efficient quantum algorithm in terms determining the shortest path between two points in a certain curved, or Riemannian, geometry. The

CREDITS (TOP TO BOTTOM): RAMASWAMY *ET AL.*; ARMAKOLAS AND KLAR

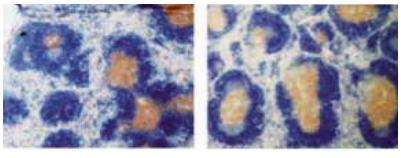
mathematical tools of Riemannian geometry can then be used to provide an understanding of quantum computation and a possible route to determine efficient quantum algorithms.

Bacteria Have Social Lives Too

Quorum sensing provides a mechanism for bacteria to monitor one another's presence and to modulate gene expression in response to changes in population density. **Camilli and Bassler** (p. 1113) review how the synchronous response of bacterial populations to small molecule autoinducers that is involved in quorum sensing confers social behavior to bacteria. Autoinducers are packaged in a variety of ways and have varying half-lives, depending on their roles. Autoinducer signals are integrated within each cell by second-messenger systems, probably by cdiGMP signaling.

Positive and Negative Transcription Regulators

The *Drosophila* Polycomb group (PcG) and Trithorax group (trxG) of epigenetic regulators maintain, respectively, either repressed or active chromosomal transcriptional states. They act via the same dual-function chromosomal elements to exert their effects. Transcription through these elements switches them from silent Polycomb response elements (PREs) to active Trithorax response elements (TREs). **Sanchez-Elsner *et al.*** (p. 1118) show that noncoding RNAs generated by PRE/TRE transcription in the ultrabithorax (Ubx) locus function to recruit the histone methyltransferase Ash1, an activator of Ubx expression. Ash1 interacts specifically with the chromatin-associated TRE noncoding RNAs. Although TRE noncoding RNAs are retained at Ubx TREs, possibly through RNA-DNA interactions, they can also act in trans to recruit Ash1 to their counterpart TREs and activate Ubx transcription.



Timely Demise and Immune Control

Apoptosis, or programmed cell death, is a fundamental means by which the immune system regulates itself. Autoimmunity develops when components of the cell death machinery, such as the cell surface receptor Fas and its ligand, are mutated or absent. Generally, this change is considered to be due to direct defects in lymphocytes, leading to their aberrant activation and proliferation. However, **Chen *et al.*** (p. 1160; see the news story by **Marx**) challenge this assumption by revealing that correctly regulated cell death of another central immune cell—the dendritic cell (DC)—is also required to maintain immune control. To prevent apoptosis, a transgene encoding a caspase inhibitor was targeted to DCs in mice, resulting in the accumulation of these cells; both in their resting state, as well as in situations of antigen-priming. As a consequence, T cells in these animals became chronically activated and dysregulated, leading to telltale signs of autoimmunity.

DNA Damage-Transcription Links

Damage to DNA in cells (like that produced by some anticancer drugs) is sensed by the cell and causes cellular responses that determine whether a cell lives or dies. **Wu *et al.*** (p. 1141; see the Perspective by **Bartek and Jiri**) provide a new link by which this signal can be conveyed from the nucleus to the cytoplasm. The protein kinase ataxia telangiectasia mutated (ATM) is activated in response to DNA damage and directly phosphorylates NEMO, one of the proteins in the IκB kinase (IKK) complex that regulates the activity of the transcription factor NF-κB. NF-κB in turn mediates signals that promote cell survival. After DNA damage, ATM was exported from the nucleus and then interacted in the cytoplasm with another protein in the IKK complex, ELKS. Activated IKK then caused activation of NF-κB–dependent transcription.

Searching for a Damaged Needle in a DNA Haystack

How does a DNA repair enzyme find a deleterious base lesion within a huge excess of normal base pairs? **Banerjee *et al.*** (p. 1153) show that a bacterial DNA glycosylase can examine an intact DNA helix, and does not need to extrude damaged base pairs. Instead, a conserved phenylalanine residue inserts into the helical stack and causes buckling at the intercalation site. The probe residue senses a deformed base within the intact helix and allows for base extrusion events only at damaged sites.

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Galileo Galilei

Italian physicist, astronomer, philosopher (1564-1642)

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Randolph M. Nesse is professor of Psychiatry and Psychology at the University of Michigan, working in the field of evolution and medicine.

Stephen C. Stearns is Edward P. Bass Professor of Ecology and Evolutionary Biology at Yale University, working in the field of evolutionary biology.

Gilbert S. Omenn is president of AAAS and professor of Medicine and Genetics at the University of Michigan, working in cancer proteomics, computational biology, and science policy.

Medicine Needs Evolution

THE CITATION OF "EVOLUTION IN ACTION" AS *SCIENCE*'S 2005 BREAKTHROUGH OF THE YEAR confirms that evolution is the vibrant foundation for all biology. Its contributions to understanding infectious disease and genetics are widely recognized, but its full potential for use in medicine has yet to be realized. Some insights have immediate clinical applications, but most are fundamental, as is the case in other basic sciences. Simply put, training in evolutionary thinking can help both biomedical researchers and clinicians ask useful questions that they might not otherwise pose.

Although anatomy, physiology, biochemistry, and embryology are recognized as basic sciences for medicine, evolutionary biology is not. Future clinicians are generally not taught evolutionary explanations for why our bodies are vulnerable to certain kinds of failure. The narrowness of the birth canal, the existence of wisdom teeth, and the persistence of genes that cause bipolar disease and senescence all have their origins in our evolutionary history. In a whole array of clinical and basic science challenges, evolutionary biology is turning out to be crucial. For example, the evolution of antibiotic resistance is widely recognized, but few appreciate how competition among bacteria has shaped chemical weapons and resistance factors in an arms race that has been going on for hundreds of millions of years. The incorrect idea that selection reliably shapes a happy coexistence of hosts and pathogens persists, despite evidence for the evolution of increased virulence when disease transmission occurs through vectors such as insects, needles, or clinicians' hands. There is growing recognition that cough, fever, and diarrhea are useful responses shaped by natural selection, but knowing when is it safe to block them will require studies grounded in an understanding of how selection shaped the systems that regulate such defenses and the compromises that had to be struck.

Evolution is also the origin of apparent anatomical anomalies such as the vulnerabilities of the lower back. Biochemistry courses cover bilirubin metabolism, but an evolutionary explanation for why bilirubin is synthesized at all is new: It is an efficient free-radical scavenger. Pharmacology emphasizes individual variation in genes encoding cytochrome P450s, but their evolutionary origins in processing dietary toxins are just being fully appreciated. In physiology, fetal nutritional stress appears to flip an evolved switch that sets the body into a state that protects against starvation. When these individuals encounter modern diets, they respond with the deadly metabolic syndrome of obesity, hypertension, and diabetes.

The triumphs of molecular biology call attention to evolutionary factors responsible for certain genetic diseases. The textbook example is sickle-cell disease, whose carriers are resistant to malaria. Similar protection against infection has been hypothesized for other disorders. Which aspects of the modern environment are pathogenic? We need to find out. Increases in breast cancer have been attributed to hormone exposure in modern women who have four times as many menstrual cycles as women in cultures without birth control. Other studies suggest that nighttime exposure to light increases the risk of breast cancer by inhibiting the normal nighttime surge of melatonin, which may decrease tumor growth. Evolution has also provided some explanations for conditions such as infertility. The process that eliminates 99.99% of oocytes may have evolved to protect against common genetic defects. And some recurrent spontaneous miscarriages may arise from a system evolved to protect against investing in offspring with combinations of specific genes that predispose to early death from infection.

These and other examples make a strong case for recognizing evolution as a basic science for medicine. What actions would bring the full power of evolutionary biology to bear on human disease? We suggest three. First, include questions about evolution in medical licensing examinations; this will motivate curriculum committees to incorporate relevant basic science education. Second, ensure evolutionary expertise in agencies that fund biomedical research. Third, incorporate evolution into every relevant high school, undergraduate, and graduate course. These three changes will help clinicians and biomedical researchers understand that both the human body and its pathogens are not perfectly designed machines but evolving biological systems shaped by selection under the constraints of tradeoffs that produce specific compromises and vulnerabilities. Powerful insights from evolutionary biology generate new questions whose answers will help improve human health.*

Randolph M. Nesse, Stephen C. Stearns, Gilbert S. Omenn

10.1126/science.1125956

*References for this editorial can be found at www.EvolutionAndMedicine.org.

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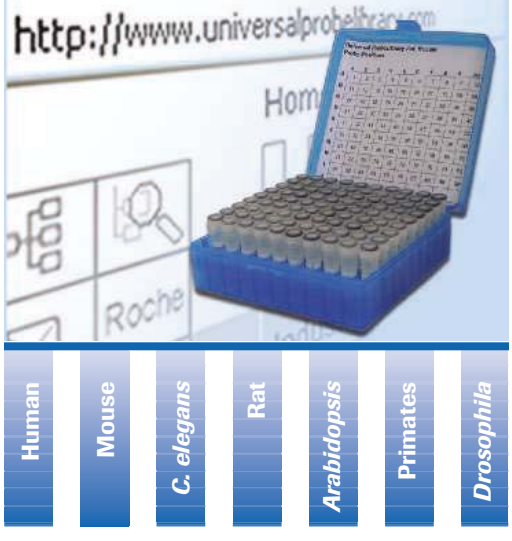

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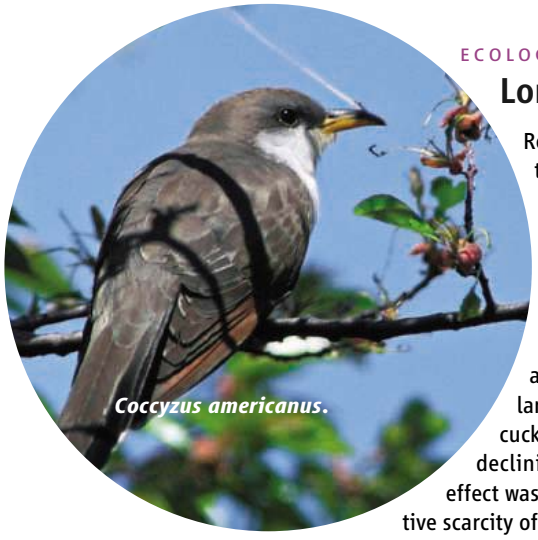

Coccyzus americanus.

ECOLOGY/EVOLUTION

Long-Term Loss of Landbirds

Recent studies have documented the effects of climate variation on the distribution and local survival of a variety of animal species. However, the effects of contemporary climate change on population density across the entire range of a species, and hence on its potential population decline, have remained mostly unexplored.

Birds are the only group of organisms for which reliable data exist over ecologically significant stretches of time. Anders and Post quantified the relationships over four decades between climatic oscillations, local temperatures, and population biology of the yellow-billed cuckoo, a North American migrant landbird, using data from the U.S. Geological Survey's Breeding Bird Survey. The cuckoo population densities across their breeding range showed a lagged effect, declining after years when the local temperatures were high. The strength of this effect was predictive of longer-term population decline, which may be caused by a relative scarcity of invertebrate prey after warmer winters. — AMS

J. Anim. Ecol. **75**, 221 (2006).

ASTRONOMY

Stellar Construction Sites

How and when did galaxies assemble all their stars? Two studies report a census of galaxies across cosmic time and the evolution of star formation rates over the universe's history. Using near-infrared and optical emission data, Kong *et al.* found that in 80% of distant large galaxies, stars formed at a prodigious rate, much more rapidly than in galaxies of similar mass today. These ancient galaxies appear to have formed all of their stars in a vigorous burst, lasting only a hundred million years.

Caputi *et al.* observed a similar pattern of exceptionally rapid star formation in old galaxies. By analyzing mid-infrared emission detected with the Spitzer space telescope, they also found evidence for the presence of complex molecules (polyaromatic hydrocarbons) in the interstellar region of these galaxies at early times. Both teams suggest that their findings favor a "cosmic downsizing" phenomenon, with galaxy formation being more active and rapid in the young universe than at present. — JB

Astrophys. J. **638**, 72; **637**, 727 (2006).

EARTH SCIENCE

Weighing Ice Sheets

Melting of the Greenland and Antarctic ice sheets is the largest potential contributor to sea level rise, but calculating the mass balance over time of such large and topographically diverse areas is difficult. Many individual measurements must be combined to create a composite picture of the whole, and techniques that track surface elevation accurately in the larger, more uniform interior sections are not as accurate when applied to the relatively narrow, high-relief coastal margins. Moreover, mass change estimates based only on elevation data do not take into account the height variations caused by compaction of the snow that covers the ice. Recent studies have documented mass loss along the margins and concurrent mass gain in the interiors, but the net effect of these compensatory processes is unclear.

Zwally *et al.* used satellite-based radar altimeters to track elevation changes for nearly all of Greenland and Antarctica over a decade. In addition to applying improved methods of data analysis, the authors incorporated estimates of density variation due to firn compaction. Their integrated assessments suggest that although Greenland is gaining mass, Antarctica is melting at a comparatively faster rate, resulting in a net rise in sea level. These conclusions differ both in sign and in magnitude from those of several other studies (for instance, see Rignot and Kanagaratnam, Reports, 17 February 2006, p. 986), leaving open the question of how to reconcile the findings. — HJS

A view of the Greenland ice sheet. Yet Do the Greenland Ice Sheet Support

J. Glaciol. **51**, 509 (2005).

CHEMISTRY

Two Rings to Bind Them All

Metallocene polymerization catalysts—two cyclic aromatic rings flanking a central metal (generally Ti, Zr, or Hf) center—have recently been optimized for the commercial production of plastics. Although heterogeneous catalysts are more widely used, the well-defined structure and ligand tunability of the metallocenes offer more rational control over the characteristics of the polymer product, particularly its stereochemistry. However, these molecular catalysts have generally been ineffective in making ultrahigh-molecular-weight polyethylene, an especially tough, resilient plastic.

Starzewski *et al.* have designed a zirconocene that overcomes this deficiency and yields polyethylene with chain molecular weights exceeding a million g/mol. They tuned the catalyst's electronic properties to favor continual insertion of ethylene monomers into the growing polymer chain and achieved the necessary >10,000:1 selectivity for chain growth over termination by linking the cyclic

Continued on page 1075

CREDITS (TOP TO BOTTOM): J. SCHUMACHER / PETER ARNOLD; J. MCCONNICO/AP PHOTO

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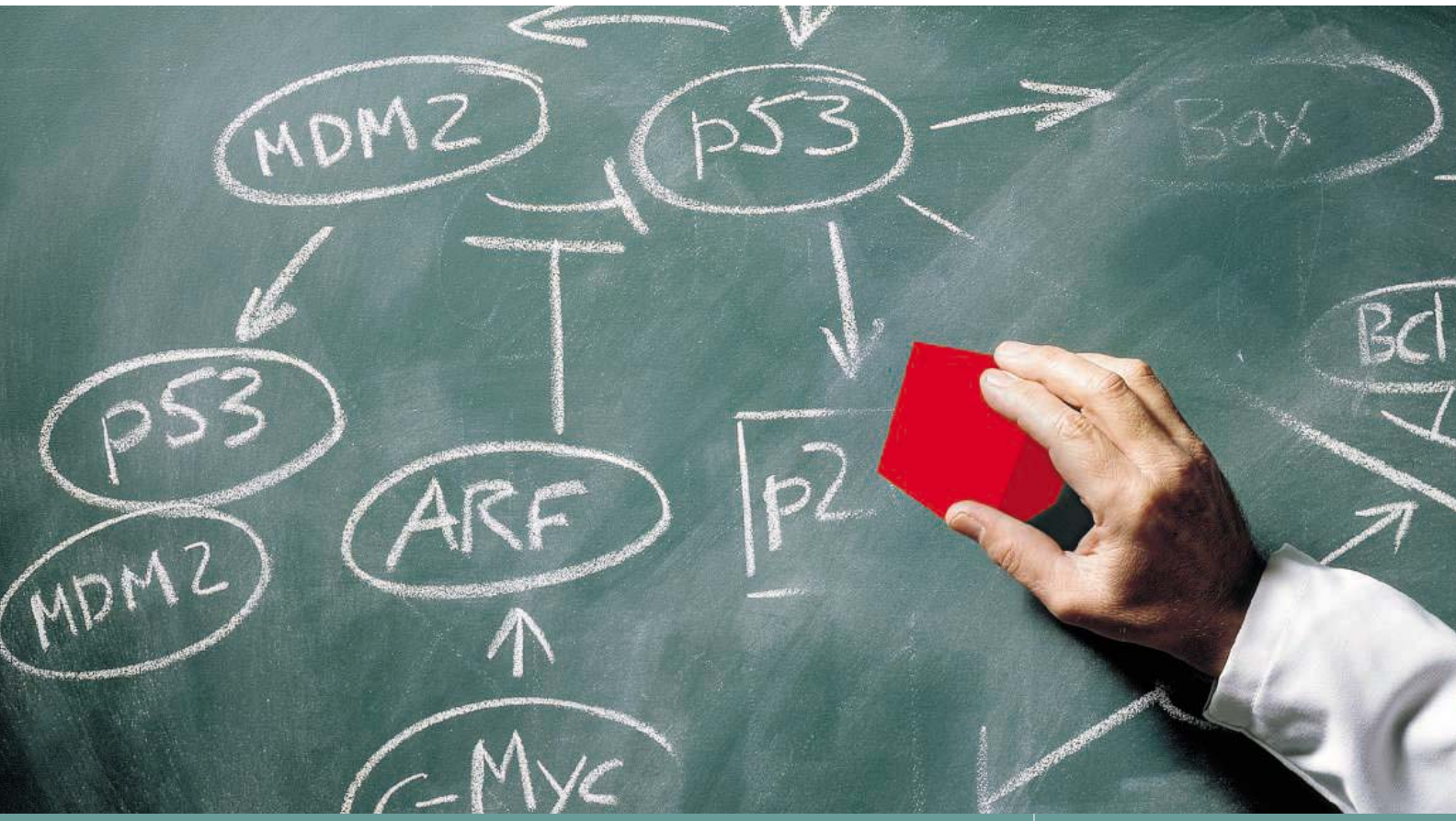

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Continued from page 1073

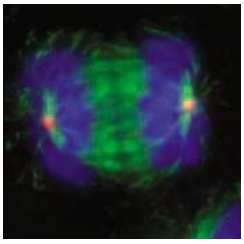
ligands around Zr through a dative bond between a phosphine on one ring and a borane on the other. — JSY

Angew. Chem. Int. Ed. **45**, 10.1002/anie.200504173 (2006).

CELL BIOLOGY

Recruitment to an Organization

Eukaryotic cells contain a dynamic array of microtubules (tubulin polymers), which play diverse roles in interphase but are dramatically rearranged into a spindle during mitosis to promote chromosome segregation. The centrosome, which is composed of a pair of centrioles and associated material, is a key organizer of microtubules and contains the γ isoform of tubulin. In mammalian cells, γ-tubulin is found in a ring-like complex together with other proteins, and Haren *et al*. characterize NEDD1, a protein of the centrosome that is associated with γ-tubulin ring structures. NEDD1 is not required for γ-tubulin ring complex assembly; in its absence, the complex is not correctly recruited to the centrosomes although NEDD1 is targeted to the centrosome even in the absence of γ-tubulin. Depletion of NEDD1 causes centrosomal defects and compromises the quality of the mitotic spindle and microtubule organization in interphase cells. Interfering with the NEDD1–γ-tubulin interaction blocks centriole duplication. Thus, it appears that NEDD1 mediates the interaction between γ-tubulin and the centrosome, which is necessary for centriole duplication and the fidelity of mitosis. — SMH



During anaphase when chromosomes (blue) separate, the mitotic spindle (green) shows NEDD1 (red) localized at the centrosome.

CREDITS: HAREN *ET AL.*, *J. CELL BIOL.* **172**, 505 (2006)

J. Cell Biol. **172**, 505 (2006).

BIOMEDICINE

Innate Immunity and Tumor Growth

The Nod-like family of receptors of the intra-epithelial cell are considered important sensors of pathogenic bacteria. Nod1 is activated by bacterial peptidoglycan and is associated with apoptotic pathways in the cell.

Using retroviral mutagenesis in a human breast cancer epithelial cell line (MCF-7), da Silva Correia *et al.* tested the possibility that the proapoptotic character of Nod1 might be involved in another context where the regulation of cell death is critical. In a Nod1-deficient MCF-7 clone, the sensitivity to tumor necrosis factor α–induced cell death and the apoptotic response to the specific Nod1 activator diaminopimelic acid were both greatly reduced. The disruption of Nod1 also resulted in an increased ability of the MCF-7 clone to generate tumors in immunodeficient mice and an enhanced sensitivity to estrogen-induced tumor growth. It will be interesting to explore how a bacterial cell wall detector is involved in regulating tumor growth and whether this might afford a therapeutic opportunity. — SJS

Proc. Natl. Acad. Sci. U.S.A. **103**, 1840 (2006).

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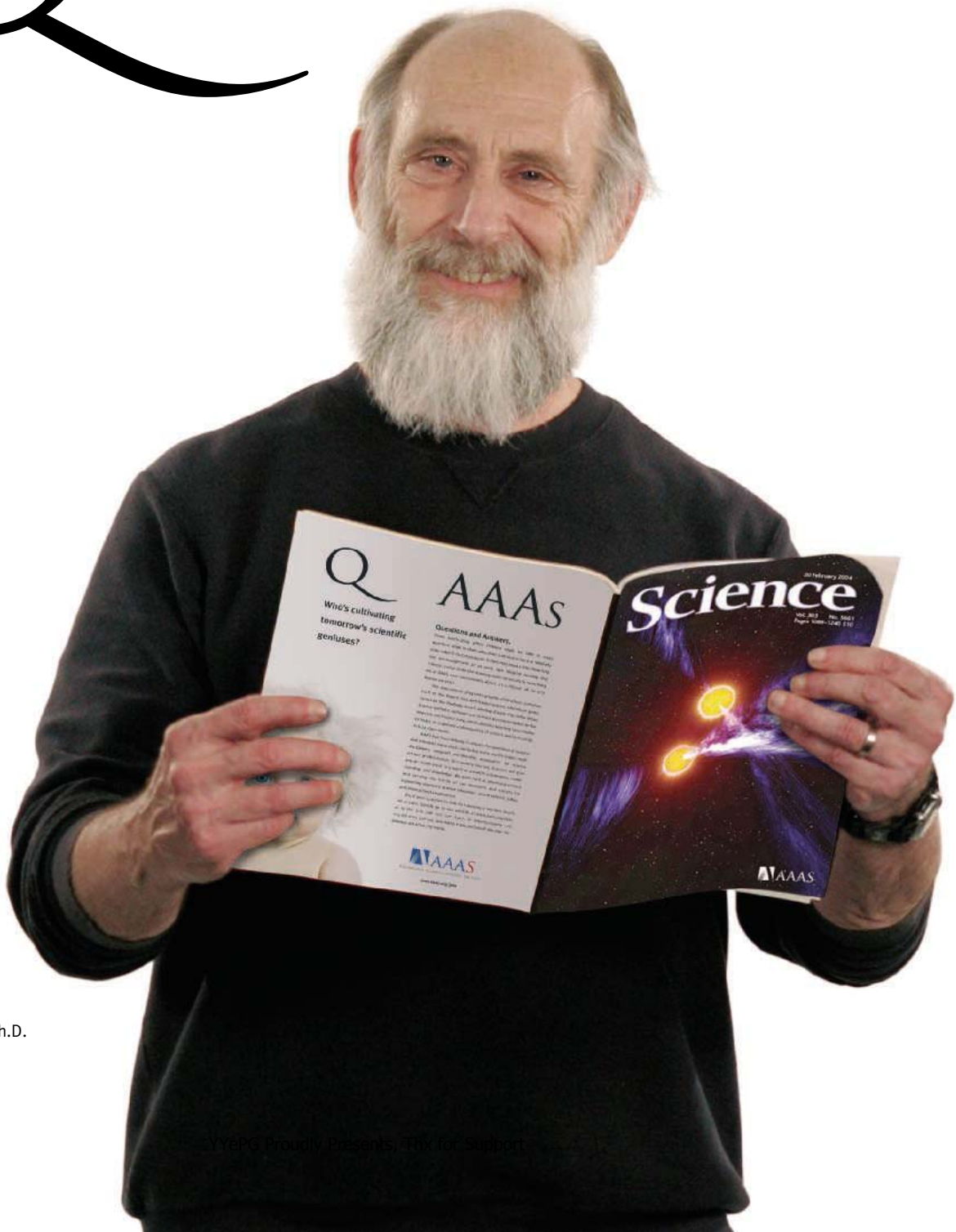
<< Targeting Downstream Repercussions

The tumor suppressor p53, which enforces cell-cycle arrest or cell death, is mutated in roughly half of malignant tumors. Nutlins are imidazoline compounds that disrupt the interaction between p53 and the E3 ubiquitin ligase MDM2 that targets p53 for degradation. Tovar *et al*. show that in 10 cell lines (representing a range of solid tumors) that express wild-type p53, adding nutlin-3a (and hence freeing p53) resulted in cell-cycle arrest although the extent of apoptosis varied. The osteosarcoma cell line SJSA-1, which has a highly amplified *mdm2* gene, was the most sensitive to nutlin-3a–induced apoptosis. To verify that *mdm2* amplification was responsible, two other osteosarcoma cell lines—MHM, which has a moderately amplified *mdm2*, and U2OS, which has a single copy of *mdm2*—were also analyzed. All three exhibited cell-cycle arrest when exposed to nutlin-3a; however, the induction of apoptosis varied with *mdm2* copy number. Microarray analysis showed that proapoptotic genes, such as *puma*, *noxa*, and *bax*, were more strongly stimulated in cells with amplified *mdm2* in response to nutlin-3a. Finally, nutlin-3a caused tumor regression in nude mice with MHM and SJSA-1 tumors and halted the growth of tumors that had normal MDM2 and p53. These results suggest that (i) nutlins may be effective clinically, especially for tumors with *mdm2* amplification and (ii) cancer cells with normal p53 may have defects in the p53 apoptotic pathway. — NRG

Proc. Natl. Acad. Sci. U.S.A. **103**, 1888 (2006).

Q

Who's opening the pipeline to new discoveries?



Leonard Susskind Ph.D.
Professor of Physics
and AAAS member

AAAs

"I started out as a plumber in the Bronx, New York. My father was a plumber. He wanted me to go to college to learn engineering so we could go into business together.

But I was no good at engineering and switched to physics. I got hooked, and quickly knew that I wanted to be a physicist. I had to break it to my father. He didn't know what a physicist was, so I said – like Einstein.

Well, I may not be Einstein but I did become a physicist. It appeals to my curiosity.

At some point I just knew I wanted to spend my life finding out how the natural world works.

I'm a member of AAAS because I believe in what it does for science and scientists. A big part of that work is in education. I think its efforts to bring on the next generation of scientists are vital for our future."

Dr. Leonard Susskind is a professor of physics at Stanford University. He's also a member of AAAS.

See video clips of this story ond others at **www.aaas.org/stories**

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See pages 102 and 103 of the 6 January 2006 issue or access www.sciencemag.org/feature/contribinfo/home.shtml

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EXHIBITS

Twisted Science

Hitler's regime distorted anthropology, psychology, and genetics to justify murdering millions of Jews and other people deemed inferior. Deadly Medicine, an online version of an exhibit at the U.S. Holocaust Memorial Museum in Washington, D.C., reviews how the Third Reich's pursuit of "racial hygiene" led to mass homicide.

The Nazis absorbed eugenic ideas that were prevalent in Germany and elsewhere, the site notes, but went further than other countries in their efforts to "strengthen the national body." From mandatory sterilization of people with schizophrenia and other supposedly inherited diseases, they moved on to euthanasia of children with birth defects and institutionalized patients. In video conversations, the exhibit's curator explores disturbing documents from the time, such as this mid-1930s poster (above) warning against the crime of "racial defilement," or mixing between Jews and non-Jews. Brief profiles describe scientists and doctors who helped shape the Reich's racial policies and in many cases resumed their careers after the war. >>
www.ushmm.org/museum/exhibit/online/deadlymedicine

RESOURCES

Language of Lava >>

Resembling bundles of licorice, this gnarled lava on Hawaii's Kilauea volcano (right) is known as pahoehoe. The plaited texture forms when the lava's crust slows or stalls but the material below keeps flowing, stretching the surface. Learn to recognize pahoehoe and other volcanic features at this illustrated glossary from the U.S. Geological Survey (USGS). Photos, drawings, and animations can help users distinguish types of volcanoes, eruptions, and ejected material. The glossary is part of the USGS Volcano Hazards Program Web site, which also offers a primer on dangers from volcanoes, information on historic eruptions, and other facts. For the latest on U.S. volcanic activity, click over to the observatories that monitor rumblings in Alaska, the Cascade Range, Yellowstone, and Hawaii. >>
volcanoes.usgs.gov/Products/Pglossary

WEB LOGS

Water Cooler Physics

For a discussion of research funding in the new federal budget, musings about scientists' public image, or a host of other opinions, click over to Cosmic Variance. At the 8-month-old blog, five physicists and astrophysicists from institutions around the United States discourse daily about their field and anything else that catches their fancy, whether it's politics or the arts. Scientific posts include heads-ups about noteworthy discoveries, such as new measurements that indicate dark matter might be warmer than predicted, and a commentary on a *New York Times* write-up of a book on astrology. Contributor Mark Trodden of Syracuse University in New York denounces the reviewer for "willful twisting of hard-won scientific progress." >>
cosmicvariance.com

TOOLS

Gene-o-Matic

This new program from the Johns Hopkins Medical Institutions in Baltimore, Maryland, is a timesaver for scientists who craft DNA sequences for genetic engineering or to decipher gene functions. Users key in a protein sequence, and GeneDesign specifies a DNA blueprint that researchers can synthesize themselves or order from a company. GeneDesign lets users customize their creations for a particular vector—a DNA snippet that ferries the sequence into cells—and for the organism they are studying. >> slam.bs.jhmi.edu/gd

IMAGES

Mollusks With Attitude

One of the newest painkillers on the market, ziconitide, comes from the sting of a snail—not the ones that demolish your cucumbers but their marine cousins, the cone snails of the genus *Conus*. The rapacious creatures subdue fish and other animals with their poison-tipped mouthparts. To help taxonomists tidy up this complicated group, Trevor Anderson and Alan Kohn of the Burke Museum of Natural History and Culture in Seattle, Washington, launched this catalog of the more than 3000 known *Conus* species. Mollusk mavens can also peruse photos and drawings of more than 600 type specimens, the original samples researchers used to delineate a species or other taxonomic group. Pioneering classifier Carolus Linnaeus consulted the *Conus marmoreus* specimen above when he named the genus in 1758. A dozen or so video clips show the predatory snails ambushing and gobbling their victims. Anderson and Kohn plan to add species accounts with range maps and other data. >>
biology.burke.washington.edu/conus

CREDITS (TOP TO BOTTOM): U.S. HOLOCAUST MEMORIAL MUSEUM; T. N. MATTOX/USGS; ALAN KOHN/BURKE MUSEUM

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2300-year-old pipe

LIGHTING UP IN VERMONT

Some are trying to make Vermont smoke-free these days, but it wasn't always thus. An analysis of residues from a pipe unearthed at a site in northern Vermont has pushed dates of the earliest tobacco use in the eastern United States back at least 500 years.

Archaeologists have been unsure about how and when tobacco spread northward from its origins in South America. Tobacco seeds from a New Mexico cave have been dated to 1040 B.C.E., but the earliest well-accepted traces in the east are some 1200 years later.

Recently, Sean Rafferty, an archaeologist at the University of Albany in New York, obtained the residues from a pipe found buried with a young woman at the site. The burial was dated to about 300 B.C.E., and gas chromatography and mass spectrography tests revealed that the residue contained nicotine. Rafferty, whose report will appear in the April *Journal of Archaeological Science*, speculates that tobacco may have been used even longer because the place of burial, known as the Boucher site, was founded about 700 B.C.E.

David Anthony, an archaeologist at Hartwick College in Oneonta, New York, says that the finding—along with others by Rafferty in West Virginia—"establishes that tobacco smoking was widespread" at the time. He agrees with Rafferty that the pipes were probably used in rituals. The Boucher site was in the Adena tradition, a culture "defined by an explosion of ritual ceremonialism," he says, and the type of tobacco used—*Nicotiana rustica*—was much stronger than that smoked today.

Fit for a Queen

Archaeologists this month announced the discovery of a hidden tomb in the Valley of the Kings near Luxor, Egypt, the first since King Tutankhamun's was unearthed in 1922. The tantalizing possibility is that the tomb is the long-sought resting place of Queen Nefertiti.

During routine fieldwork, a team led by Otto Schaden, an Egyptologist at the University of Memphis, Tennessee, came upon a 4-meter-deep stone shaft leading to a chamber holding mummies of several adults and a child. The style of the brightly colored sarcophagi dates them to about 1330 B.C.E., says Betsy Bryan, an Egyptologist at Johns Hopkins University in Baltimore, Maryland. "What is most exciting," adds Bryan, "is [the tomb] offers a glimpse at the strangest period in Egyptian history": the late 18th Dynasty, when the heretical King Akhenaten brought a brief period of sun-worshipping monotheism to Egypt. His wife Nefertiti acted as king after his death. Because Akhenaten's religion considered death final, "one theory is that Nefertiti's body was buried in the Valley of the Kings to make sure she had an afterlife," says Bryan.

Identification of the mummies will be tough. If no written record is found, Bryan says it might be useful to reconstruct their faces and compare them to an existing bust of the queen. "The tomb is most likely that of an elite but nonroyal group," says Stephen Buckley, an Egyptologist at the University of York, U.K. But even without the queen, he says, it is "an exciting discovery" that will keep researchers busy for years to come.

ORIGINATING LIFE

An experiment at a Russian volcano has thrown cold water on the theory that life on Earth began with organic materials in a puddle of hot water.

The notion that the first biochemical steps toward life occurred 4 billion years ago at high temperatures is supported by lab experiments, as well as some genetic evidence that life started with microbes like those found in hot springs and around hydrothermal vents.

Biophysicist David Deamer of the University of California, Santa Cruz, and his colleagues decided to see if they could create a semblance of life in a pool of water heated by volcanic activity on Russia's Kamchatka Peninsula. They added a "primordial soup" of proteins, DNA, and cell membranes. "Darwin proposed that life started in 'a warm little pond.' … We are testing his theory in 'a hot little puddle,' " Deamer related at a meeting of the Royal Society in London last week.

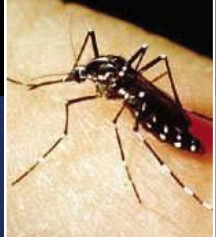
The soup ingredients largely disappeared in a few hours. The molecules had stuck to the clay that lined the pool and couldn't assemble. "It is an interesting experiment," says chemist James Ferris of Rensselaer Polytechnic Institute in Troy, New York, but he suggests that the puddle was too hot and acidic. Deamer plans to repeat the study at a puddle on a Hawaiian volcano where clay may be less of a problem.

Mac Discrimination

Macintosh computer users have yet another reason to complain about Microsoft founder Bill Gates: They're shut out of the National Institutes of Health's (NIH's) new online grant submission system.

As a first step to a paperless process, NIH in December began accepting submissions for small business grants through Grants.gov. But that site uses an electronic form developed by a company, PureEdge Solutions, that only works on a Microsoft Windows platform. A temporary fix for Mac users—Windows-emulating software—hasn't worked out well, according to frustrated university officials quoted in a 13 February story in *The Washington Post*. PureEdge says a Mac version of the forms will be ready by November.

NIH, besieged by complaints about the Mac flaw and other problems, has already announced that it is delaying electronic submissions for R01 research grants from October 2006 to February 2007.



Chikungunya on a rampage
1085

Why the cochlea looks like a shell
1087

SCIENCE AND RELIGION

Evangelicals, Scientists Reach Common Ground on Climate Change

As chief lobbyist for the National Association of Evangelicals (NAE), Reverend Richard Cizik never imagined spending a day with a bunch of climate-change scientists, much less leaving such a meeting convinced that working to mitigate global warming was consistent with his religious beliefs. But Cizik says a 2002 gathering in Oxford, U.K., was "a conversion … not unlike my conversion to Christ." And he's not alone: This month, 86 influential leaders in the U.S. Christian evangelical movement came out for "national legislation requiring … economy-wide reductions" in carbon emissions. Quoting the Bible on the need to protect God's creation, the statement says that climate shifts "will hit the poor the hardest."

Warming trend. From left, Richard Cizik of the National Association of Evangelicals talks with ecologist Calvin DeWitt and atmospheric scientist John Houghton.

The 8 February statement (www.christiansandclimate.org) is seen as an important boost for supporters of mandatory controls on U.S. greenhouse gas emissions. The Evangelical Climate Initiative also represents the fruits of a 5-year effort by a handful of scientists, most of them devout Christians, to find common ground with an influential Republican constituency that is often an implacable enemy in science policy debates. The signers include the president of Wheaton College, a preeminent evangelical school in Illinois, and Reverend Rick Warren, pastor of an 85,000-member church in Lake Forest, California, and author of the bestseller *The Purpose Driven Life*. "What's going on here is peacemaking at its most basic level between the religious and scientific worldview," says forester Jim Furnish, former deputy chief of the U.S. Forest Service and an organizer of the effort.

The 2002 Oxford meeting that advanced the cause was organized by John Houghton, former co-chair of the science assessment for the 2001 Intergovernmental Panel on Climate Change (IPCC) report, and ecologist Calvin DeWitt of the University of Wisconsin, Madison. As evangelicals whose speeches often quote the bible, the scientists hoped to raise awareness of global warming on both sides of the Atlantic. "U.S. evangelicals' information [on global warming] had predominantly been from the active misinformation campaign you have in [the U.S.]," Houghton says. ▶

NUCLEAR PHYSICS

Accelerator Delay Stuns U.S. Scientists

Plans for a nuclear physics facility that would mimic stellar explosions have been pushed back 5 years, U.S. Secretary of Energy Samuel Bodman told Congress last week. U.S. researchers developing the Rare Isotope Accelerator (RIA) say the new timetable could put other countries in the lead.

"This catches me completely by surprise, and it's quite alarming," says Konrad Gelbke, director of the National Superconducting Cyclotron Laboratory at Michigan State University in East Lansing. Robert Tribble, a physicist at Texas A&M University in College Station and chair of the Department of Energy's (DOE's) Nuclear Science Advisory Committee, says the delay "will be a significant loss for the field."

Researchers at Michigan State and Argonne National Laboratory in Illinois are vying for RIA. Meanwhile, researchers at the Japanese laboratory RIKEN in Wako and the German laboratory GSI in Darmstadt are developing their own machines. "They'll get a first crack at the science while we're standing on the sideline," says Argonne's Robert Janssens. But he adds that RIA's ability to generate beams 10 to 100 times more intense than those at other facilities makes it still worth the wait.

Generating unstable nuclei normally produced only in stellar explosions, RIA might allow researchers to develop a comprehensive theory of the nucleus. But DOE couldn't fit the $1 billion facility into its 2007 science budget, Bodman told the House Science Committee—even if Congress approves the president's request for a 14% increase (*Science*, 10 February, p. 762).

DOE will continue to spend $5 million to $6 million a year on research and development and will aim for a preliminary engineering design by 2011, Bodman told legislators in response to a question from Representative Joe Schwarz (R–MI). "So in effect, the project will be put off for 5 years," he said. "I know that's not happy news, … but those are the facts."

Conceived in 1999, RIA tops nuclear scientists' wish list and stands third on a 2003 ranking of 28 major facilities DOE hopes to build (*Science*, 14 November 2003, p. 1126). In 2004, it passed the first of five major reviews. But last year, DOE canceled a "request for proposals," and the White House Office of Management and Budget ordered a review of its scientific potential. That review is now being conducted by the National Academies' National Research Council. NRC's Donald Shapero says the committee may issue an interim report this spring.

–ADRIAN CHO

CREDIT: KARA BALL

The meeting included sessions by top climate researchers, policymakers, and theologians. "Does [Scripture] not mean that we are called to those places where creation is most threatened?" Reverend John Paarlberg of New York's First Church in Albany asked the crowd in one of several speeches.

The link between environmentalism and Christian faith was underscored during a 2004 "creation-care" retreat at a Christian conference center in Maryland. The Wynnewood, Pennsylvania–based Evangelical Environmental Network, headed by Reverend Jim Ball, was among the organizers of the gathering, which produced a pledge from some 30 influential evangelicals to fight climate change.

Those two gatherings helped establish crucial trust, Ball says. But more work was needed before the 86 leaders were ready to sign a statement last year that "human-induced climate change is real." Rightward political leanings—"Evangelists aren't tree-huggers," one Christian biologist says—were one obstacle. So too was what DeWitt calls science's "connection with evolution."

So Ball enlisted devoutly religious researchers, mailing out copies of a statement on climate change signed by 50 evangelical scientists, along with a DVD of a speech by Houghton to NAE board members last March. "It's been critical to have these leaders see that the science of the IPCC was overseen by an evangelical," says Ball. "It was easier for them to trust the information."

The group now plans to spread the word among missionaries, Christian colleges, and churches. In doing so, however, they will confront a small group of climate change contrarians attempting to rebut their arguments. The group has already helped persuade NAE to veto the idea of Cizik signing his name or lending the organization's support to this month's statement. "Manmade global warming is a theory and not a scientific observation," writes meteorologist Roy Spencer of the University of Alabama, Huntsville, on the Web site of the group, which calls itself the Interfaith Stewardship Alliance.

Spencer alleges that problems with sampling, flaws in climate models, and incomplete understanding of global weather undermine the mainstream view. "Instituting mandated CO_2 cuts now will hurt the global economy, affecting the poor first," he says. Members include church leaders who have previously urged evangelicals not to take stands on certain environmental issues and conservative political heavyweights such as Focus on the Family's James Dobson and Charles Colson of Prison Fellowship Ministries.

Supporters say the successful coalition around global warming could point the way toward finding common ground on other issues, such as fetal health and mercury contamination, on which the public is divided. "We need to talk rather than draw lines in the sand," says Bishop George McKinney of the Stephens Church of God in Christ in San Diego, California.

–ELI KINTISCH

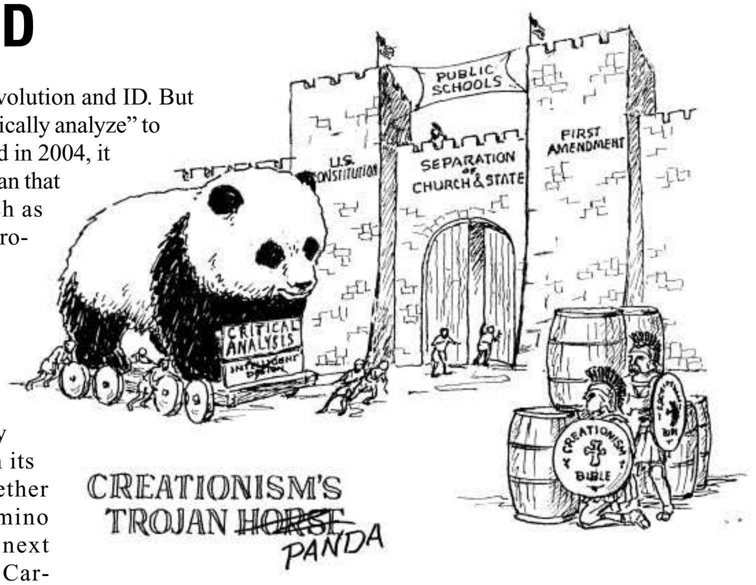
SCIENCE AND RELIGION

Ohio School Board Boots Out ID

Scientists are hailing the demise of an attempt in Ohio to sneak intelligent design (ID) into the public school science curriculum under the guise of a "critical analysis" of evolution. Last week, the Ohio Board of Education voted 11–4 to strike the words from its curriculum guidelines along with a creationist-inspired study guide. Evolution supporters called it a "stunning victory" and cited the influence of the December court ruling against the Dover, Pennsylvania, school board in the first test case of injecting ID into biology classes (*Science*, 6 January, p. 34).

"Some of my colleagues have changed their perspective" after realizing that the lesson plan "did indeed contain elements of ID that was not apparent to them before," says Robin Hovis, a Republican board member who opposed the plan. Virgil Brown, who originally supported the plan, says he changed his stance after he realized the language "was supportive of ID." Martha Wise, who spearheaded the vote, says the outcome reflects a "sea change" in the 19-member board—a change aided by the recognition following the Dover case that "it might be a legal problem that would cost Ohio millions of dollars."

In 2000, the board rejected a proposal for a "two-model approach" in which students would learn about both evolution and ID. But 2 years later, it added "critically analyze" to the evolution standard, and in 2004, it adopted a model lesson plan that suggested activities such as dividing up classes for pro- and antievolution debates.



Barring the door. Scientists see school board vote as an important defense against creationist tactics.

Many see the Ohio vote as a severe blow to attempts to cloak ID under the guise of "teaching the controversy." Kansas is now the only state with this phrase in its science standards. Whether Dover has set off a domino effect may be clearer next month when the South Carolina board of education meets to consider adding to its science standards a statement that students should be able to "investigate and critically analyze aspects of evolutionary theory."

Officials at the Discovery Institute, a Seattle, Washington–based think tank for ID, did not respond to a request for comment. A press release claims the school board had been "bullied" into "censoring teaching of evolution" and cites a recent Ohio poll indicating that 75% of the respondents believe ID should be taught along with evolution. But biologist Patricia Princehouse of Case Western Reserve University in Cleveland, Ohio, says anyone can play the survey game. Another recent poll, she says, showed that 84% of the respondents had never heard of ID.

–CONSTANCE HOLDEN

INFLUENZA

New Study Casts Doubt on Plans for Pandemic Containment

An audacious global plan to stop future influenza pandemics in their tracks, adopted by the World Health Organization (WHO), may be flawed, researchers say in a new paper in the *Public Library of Science* (*PLOS*) *Medicine*. The plan, based on containing an epidemic where it first erupts, may initially work, they write, but later-emerging pandemics would likely overwhelm it. But scientists who published studies last year supporting the containment strategy say it's the new study that is flawed.

Mathematical models published in August in *Nature* and *Science* (12 August 2005, p. 1083) predict that by dispensing huge quantities of antiviral drugs to the area where human-to-human transmission of a new influenza virus begins—the prelude to a pandemic—and enacting rigorous quarantine measures, it might be possible to nip a pandemic in the bud. On 27 January, WHO published the first draft of such a "rapid response and containment" protocol, which the agency says is worth a try, despite the nightmarish logistics.

The scheme may halt the first pandemic, write Marc Lipsitch and colleagues at Harvard School of Public Health in Boston and Carl Bergstrom of the University of Washington (UW), Seattle, in the *PLOS Medicine* paper. The problem is that, just as a parched forest rarely sees just one brushfire, if one pandemic virus emerges, it's likely that another will pop up somewhere else soon. And the more this happens, the more likely one of the containment efforts will fail, especially because, like fire brigades, the world would run out of manpower and resources.

Not that containment at the source shouldn't be tried, Bergstrom hastens to add. Even if it succeeds just once or twice, containment "would buy time, which is incredibly useful," he says, because scientists and policymakers would better understand their future foe. But in the end, the team's models show, the containment strategy may buy just a few years.

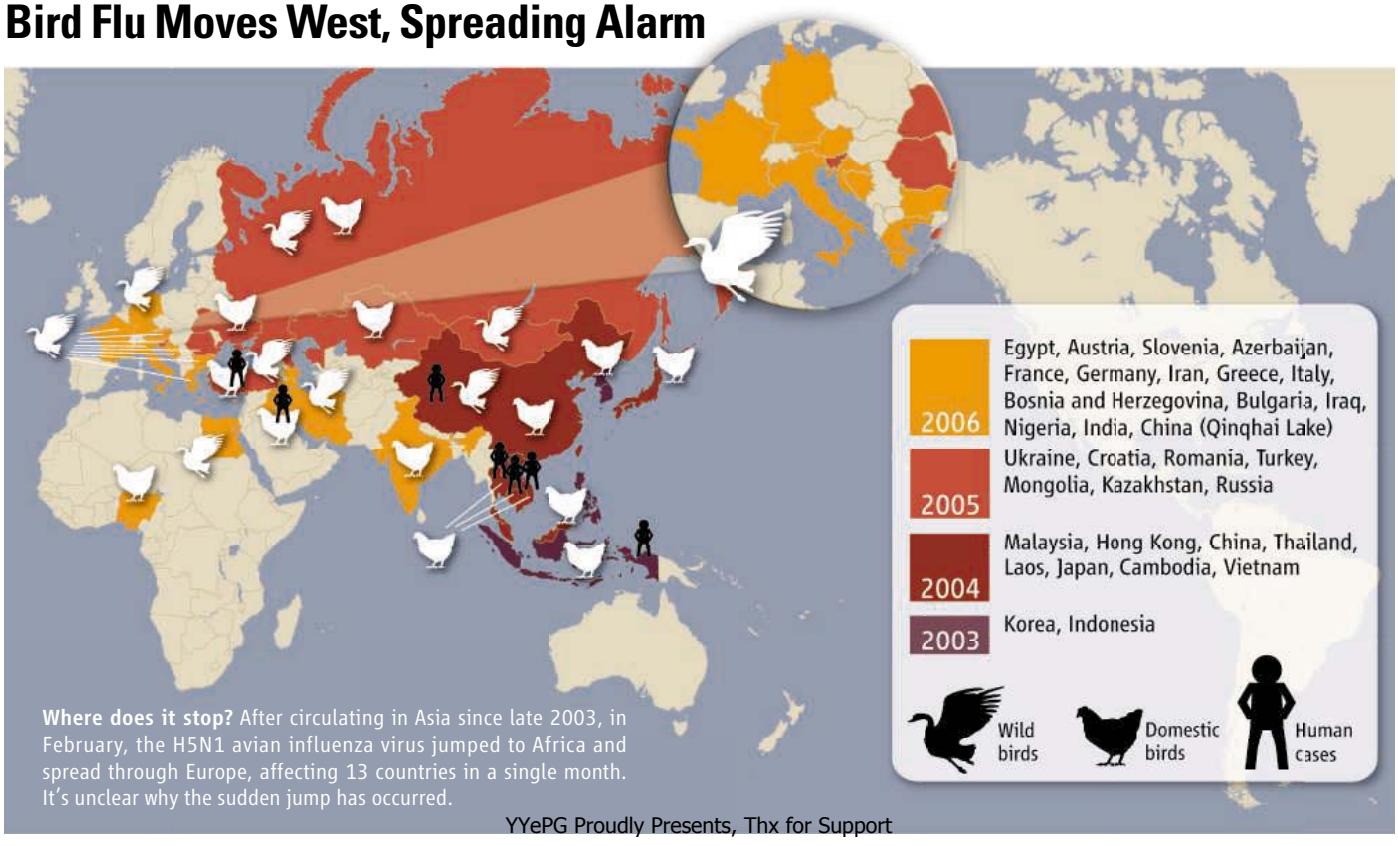
"I don't agree with that argument at all," counters Ira Longini, lead author of last year's *Science* paper. Longini, who just moved his group from Emory University in Atlanta, Georgia, to UW, believes the emergence of a pandemic virus in a human host—through mutations or recombination with a human flu virus—is and will remain a very rare event. The arrival of one pandemic virus doesn't mean the next one is around the corner, he says, noting that SARS and the "swine flu" that appeared at Fort Dix, New Jersey, in 1976 prove the point: Both emerged once and never again.

WHO influenza expert Michael Perdue agrees and says the new paper won't cause WHO to reconsider its strategy. If pandemic viruses emerged that easily, he says, the world would have seen pandemics more often, or past pandemics would have started simultaneously at several locales.

Neil Ferguson of Imperial College London, who led the team that produced last year's *Nature* paper, says he even cautioned Bergstrom and colleagues that publishing their paper might sap international enthusiasm for the containment strategy, which, he points out, is the only hope for countries too poor to stockpile their own drug and vaccine caches. But Jeremy Berg, director of the U.S. National Institute of General Medical Sciences, which funded all three studies, says that "policymakers need to weigh the arguments in this paper, too." If the controversy illustrates anything, Berg says, it's that we still know very little about how pandemics start.

–MARTIN ENSERINK

Bird Flu Moves West, Spreading Alarm



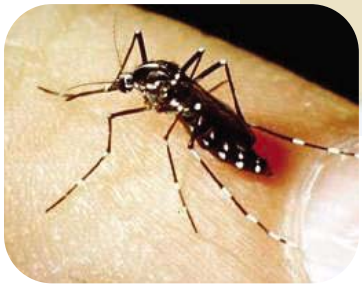
Where does it stop? After circulating in Asia since late 2003, in February, the H5N1 avian influenza virus jumped to Africa and spread through Europe, affecting 13 countries in a single month. It's unclear why the sudden jump has occurred.

SOURCE: OIE; ILLUSTRATION: K. BUCKHEIT/*SCIENCE*

INFECTIOUS DISEASES

Massive Outbreak Draws Fresh Attention to Little-Known Virus

PARIS—A French island in the Indian Ocean is reeling from an explosive outbreak of a little-known viral disease. On 17 February, the French National Institute for Public Health Surveillance said that an estimated 110,000 residents of Réunion, population 770,000, had been infected with the chikungunya virus—almost 22,000 of them between 6 and 12 February alone. Chikungunya, which is spread by mosquitoes, is rarely fatal—of 52 patients who died, all but one were suffering from other diseases as well—but it can cause high fevers, rashes, and excruciating joint and muscle pains.



Unusual suspect. Until now, the Asian tiger mosquito was not thought to be an efficient vector for the chikungunya virus, which has crippled the French island of Réunion.

"It's a massive outbreak, it's absolutely alarming," says Stephen Higgs of the University of Texas Medical Branch in Galveston, one of a few dozen researchers around the world who study chikungunya, a member of the alphavirus genus that also includes rarities such as eastern equine encephalitis and western equine encephalitis. The epidemic has triggered a wave of activity in French labs to address scientific gaps; it could also breathe new life into a vaccine candidate developed by the U.S. Army that has been languishing for almost a decade.

Chikungunya—often shortened to "chik" by scientists—is a Swahili word that means "that which bends up," a reference to some victims' inability to walk upright. The disease is known to occur in large parts of Southeast and South Asia, as well as in Africa. Preliminary sequencing of virus isolates from Réunion at the Pasteur Institute in Lyon suggests that the virus was imported from East Africa, says Pasteur virologist Nathalie Pardigon. Other Indian Ocean islands—including Mauritius, the Seychelles, and the Comoros—have also have seen cases, although far fewer.

It's unclear why the outbreak is so ferocious. One factor, says virologist Charles Calisher of Colorado State University in Fort Collins, may be that the virus is hitting Réunion for the first time, so almost no one has resistance. The mosquito species implicated as the main culprit in Réunion—*Aedes albopictus*, also known as the Asian tiger mosquito—was not believed to be a very efficient chikungunya vector, says Pasteur entomologist Paul Reiter, because it bites many different species. But perhaps it has acquired a particular taste for humans in Réunion, he adds.

Although doctors can treat the symptoms with painkillers and anti-inflammatories, there are no specific drugs against chikungunya. Nor is there a vaccine. The most promising candidate thus far has been an attenuated virus, developed in the 1980s by researchers at the U.S. Army Medical Research Institute for Infectious Diseases in Fort Detrick, Maryland. Although a clinical trial in 73 volunteers, published by Robert Edelman of the University of Maryland and colleagues in 2000, showed that the vaccine triggered neutralizing antibodies, development fell flat because of a lack of money, says David Vaughn, who heads the infectious disease program at the Army's Medical Research and Materiel Command. The Réunion outbreak is "an opportunity to reactivate the research effort and to bring the vaccine to licensure," Vaughn wrote in an e-mail.

A spokesperson for French health minister Xavier Bertrand confirms that the French government is in conversations with the U.S. health and defense departments. But much more work is needed on the vaccine, he cautions—for instance, to investigate side effects, such as joint pains, which developed in some vaccinees in the clinical trial.

To address the questions, the French government announced a broad research program last week, to be carried out by multiple institutes, and including basic virology, antiviral drugs and other treatments, vaccines, and mosquito ecology and control. On Monday, it also installed a panel to coordinate the battle, chaired by Antoine Flahault, head of the public health department at the Tenon Hospital in Paris.

YYePG Proudly Presents, –MARTIN ENSERINK

CREDIT: (PHOTO) BASTIAAN DREES, TEXAS COOPERATIVE EXTENSION

Harvard President Steps Down

Lawrence Summers, the economist who in 5 years as president of Harvard University became a lightning rod for controversy, resigned 21 February amid a faculty rebellion spurred by the resignation of Arts and Sciences Dean William Kirby. The faculty at Harvard's largest school was preparing a second no-confidence vote; the first came last March after Summers's comments about women in science and his handling of other issues (*Science*, 28 January 2005, p. 492). Summers won respect for his support for research, including plans for a new science hub to be built in nearby Allston. "My greatest hope is that the University will build on the important elements of renewal that we have begun over the last several years," Summers said in a statement.

Summers, who will step down 30 June, plans to remain on the faculty as a professor. Derek Bok, who led the university from 1971 to 1991, will be interim president.

–JENNIFER COUZIN

NOAA to Navy: Sssshhh

The U.S. Navy should turn down the volume of its proposed sonar training range, says the National Oceanic and Atmospheric Administration (NOAA), which publicly released its comments on the Navy's plans last week. The Navy wants to build a sonar training facility off the North Carolina coast, and last October it concluded that the sonar would not harm marine mammals. NOAA disagreed, citing the risk of driving beaked whales and other marine mammals onto beaches. Also, NOAA said, the endangered North Atlantic right whale has been sighted nearby. The Navy is now reviewing NOAA's concerns and more than 300 substantive public comments; the final report is expected in the fall.

–KATHERINE UNGER

Scripps Lands on Jupiter

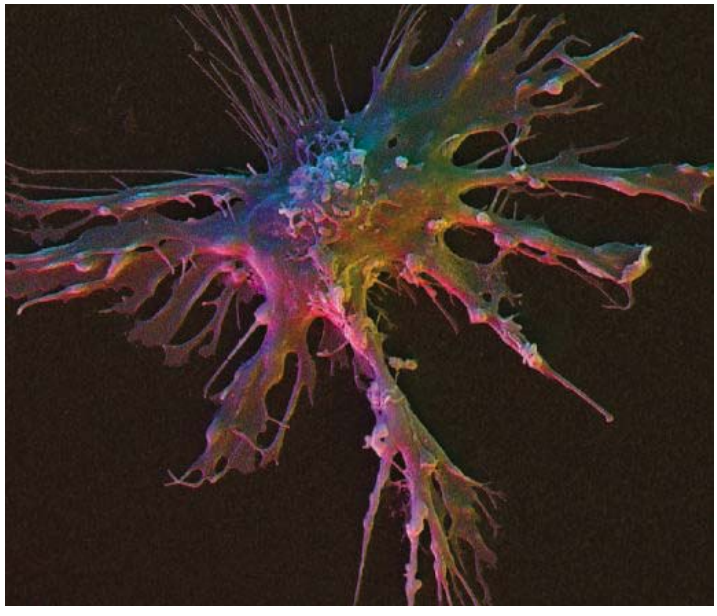
Capping a two-and-a-half-year battle over siting, officials in Palm Beach County, Florida, voted last week to approve plans by the Scripps Research Institute to build an East Coast campus in Jupiter, Florida. A suit by environmentalists had prevented the San Diego, California–based research powerhouse from building on its original choice, a site near wetlands. So Scripps officials turned to the Abacoa campus of Florida Atlantic University, where more than 160 Scripps researchers are temporarily housed.

–ROBERT F. SERVICE

IMMUNOLOGY

Foiled Dendritic Cell Suicide May Lead to Autoimmunity

When the immune system malfunctions, it can become a turncoat, attacking the body's own tissues. Such autoimmune attacks underlie many diseases, including juvenile-onset diabetes. Immunologists trying to understand these attacks have long focused on overactivity of the T lymphocytes of the immune system. New results now point to a key role for another type of cell: dendritic cells.



Autoimmune culprit? Abnormal accumulation of dendritic cells such as this one may contribute to the tissue damage of autoimmunity.

Dendritic cells activate lymphocytes to fight infection. They then die by a form of cell suicide called apoptosis, possibly cutting the risk of an autoimmune attack. On page 1160, Min Chen, Jin Wang, and their colleagues at Baylor College of Medicine and M. D. Anderson Cancer Center in Houston, Texas, report that blocking that apoptotic death in mice leads to dendritic cell buildup and the development of autoimmune symptoms.

Immunologist Roland Tisch of the University of North Carolina, Chapel Hill, says the finding has "important implications regarding the initiation or progression of autoimmunity." It also points to dendritic cells as possible targets for therapies aimed at treating autoimmune diseases.

Previous work by several groups had suggested that dendritic cell malfunction might be involved in autoimmune disease. In the late 1990s, for example, Wang, who was then a postdoc in Michael Lenardo's lab at the National Institute of Allergy and Infectious Diseases in Bethesda, Maryland, looked for mutations that might cause a human autoimmune disease characterized by excessive immune cell accumulation. In a few such cases, he and his colleagues found mutations in the gene for caspase 10, a protein-splitting enzyme activated during apoptosis to bring about cell destruction. Dendritic cells bearing those mutations underwent less apoptosis than normal and built up in the patients.

In the current work, the Baylor group genetically engineered mice to produce a caspase inhibitor called p35 in their dendritic cells. Fewer modified dendritic cells died when exposed to an apoptotic stimulus, and the cells accumulated in the animals. The mice also showed classic signs of autoimmunity such as production of antinuclear antibodies and antibody deposition in the kidneys. Lenardo says that the results make a "far more compelling argument" that defective dendritic cell apoptosis plays a role in autoimmunity than the previous human work.

Exactly how that might happen remains a mystery, however. Wang suggests that dendritic cell buildup causes persistent lymphocyte activation but concedes that "we don't know the details" of the mechanism—an issue that must be resolved before any therapeutic applications are possible.

–JEAN MARX

SCIENTIFIC CONFERENCES

U.S. Caps Number of AIDS Researchers at Toronto Meeting

The Bush Administration is again limiting attendance by federal researchers at the world's largest AIDS meeting, triggering an outcry among scientists.

The policy, from the Department of Health and Human Services (HHS), is in line with a congressional cap on the number of employees from certain agencies who can attend meetings outside the United States this year. It affects AIDS researchers at the National Institutes of Health (NIH) and the Centers for Diseases Control and Prevention (CDC) headed to the XVI International AIDS Conference in Toronto in August. Similar limits imposed 2 years ago before the same meeting in Bangkok set off charges of political interference in science.

"I lament this decision, and I think it's shortsighted," says McGill University researcher Mark Wainberg, past president of the International AIDS Society, lead sponsor of the Toronto conference. "It really doesn't make sense at all to apply these criteria to a meeting taking place on the U.S.'s doorstep."

The International AIDS Conference has sparked controversy in the past. The heckling of former HHS Secretary Tommy Thompson at the 2002 meeting in Barcelona upset congressional Republicans, who questioned sending 236 HHS staffers and spending $3.6 million on the conference. In 2004, the department imposed a 50-person limit for the Bangkok meeting, leaving some NIH and CDC scientists unable to present papers that had been accepted (*Science*, 23 April 2004, p. 499).

Similar caps will apply to the Toronto meeting, according to a 16 February memo from the State Department's Office of the U.S. Global AIDS Coordinator (OGAC), which runs the $15 billion President's Emergency Plan for AIDS Relief. The memo cites a 50-person limit for scientific presentations, [illegible] meeting in language setting current spending levels for several agencies, including State. The memo says a separate limit of 50 staff members will apply to HHS, with NIH getting 25 slots and CDC 20. Before the memo, NIH alone had planned to send 77 staffers. The decision was based on making the best use of overall U.S. resources for AIDS and "who needs to be there," says an OGAC official.

According to NIH sources, HHS has not objected to sending a larger number of NIH staff to other major international gatherings since 2004, including AIDS meetings. And hope springs eternal that HHS will reconsider. Helene Gayle, president of the International AIDS Society and the incoming president of CARE USA, hopes "a balance can be struck" between fiscal stewardship and "assuring the important work done by employees of HHS can be shared with the global community."

–JOCELYN KAISER

CREDIT: DAVID SCHARF/PETER ARNOLD

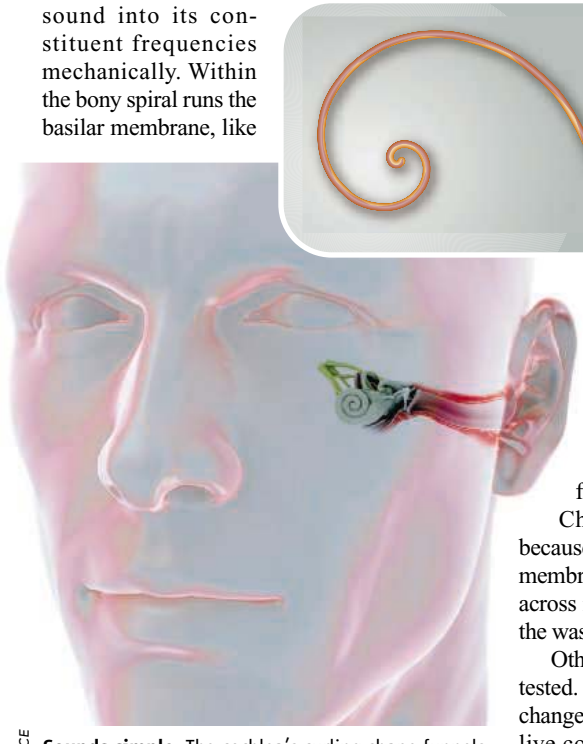
PHYSIOLOGY

Math Clears Up an Inner-Ear Mystery: Spiral Shape Pumps Up the Bass

For decades, researchers have wondered why a mammal's sound-perceiving organ, called the cochlea, coils like a seashell. Now, a team of auditory researchers has come up with a calculation that sounds to their colleagues like an answer: The spiral shape increases sensitivity to low-frequency vibrations.

Other researchers had tried and failed to find a reason for the delicate coiling, other than to save space in the skull, says Darlene Ketten, a neuroethologist at the Woods Hole Oceanographic Institution (WHOI) in Massachusetts and Harvard Medical School in Boston. "This is a new one that seems to make a great deal of sense," Ketten says.

The cochlea breaks sound into its constituent frequencies mechanically. Within the bony spiral runs the basilar membrane, like a road curving ever more tightly to one side. From the outer end to the inner end of the spiral, the membrane becomes more flexible and will oscillate up and down at progressively lower frequencies. So when vibrations of a particular frequency slosh fluid above and below the membrane, ripples run down the membrane, and it wiggles most dramatically in the place where its frequency matches the frequency of the sloshing. That makes nerve-triggering "hair cells" atop the basilar membrane brush against another membrane, producing the sensation of a tone.



Sounds simple. The cochlea's curling shape funnels energy (*inset*, purple) toward the outside of the spiral, making the ear more sensitive to low frequencies.

CREDIT: GETTY IMAGES/3D4MEDICAL.COM; (INSET) P. HUEY/*SCIENCE*

But researchers had no evidence that the cochlea's shape affected its function. Mathematical models had shown that the spiral shape affects neither where the basilar membrane oscillates in response to a given frequency nor how much it moves. The new analysis reveals a subtle but important effect after all, report applied mathematician Daphne Manoussaki of Vanderbilt University in Nashville, Tennessee, biophysicist Richard Chadwick of the National Institute on Deafness and Other Communications Disorders in Bethesda, Maryland, and colleagues.

The spiral's increasing curvature shunts energy toward the outer wall of the spiral, just as orange cones on a highway might divert cars into the right lanes on a left curve, the researchers calculate. So deep within the spiral where low pitches are detected, the outer edge of the membrane moves more than the inner edge, producing a twisting motion, they report in a paper to be published in *Physical Review Letters*. The twisting should increase the flow of fluid across the tufted hair cells and stimulate them more, increasing sensitivity to low-frequency sound by as much as 20 decibels.

The researchers suspected that the coiling did something, but "we had no idea it would be related to low-frequency" perception, Manoussaki says. Chadwick says others missed the effect because they analyzed the motion of the basilar membrane only along its centerline or averaged across its width. "They threw the baby out with the wash," he says.

Other researchers are eager to see the finding tested. "The obvious thing to do is to go in and change the curvature and see what happens" in a live cochlea, says William Brownell, a biophysicist at Baylor College of Medicine in Houston, Texas. Christine Petit, a molecular physiologist at the Pasteur Institute and the Collège de France in Paris says the idea can also be tested by comparing different species. "What would be fantastic would be to show a correlation between the capacity to detect low-frequency sound and the radius of curvature," she says.

Manoussaki, Chadwick, and WHOI's Ketten have begun such comparative analyses. And preliminary data are "right on the money," Chadwick says. That must come as music to the ears of those who have puzzled over the cochlea's curious curl.

YYePG Proudly Presents, Thx for Support

—ADRIAN CHO

California Researchers Have Day in Court; Academy Ponders

With the Bush Administration keeping the federal government on the sidelines, other groups are jumping into the breach to set policies on the use of embryonic stem cells in research. On 27 February, a trial in Alameda County Superior Court was scheduled to address whether the California Institute for Regenerative Medicine (CIRM) violates the state constitution. CIRM is expected to prevail, but the inevitable appeals are likely to take at least a year, delaying the sale of bonds to raise the $3 billion specified in the state initiative passed in November 2004.

Meanwhile, on 10 February, CIRM's governing board adopted policies regulating egg acquisition and intellectual property that will eventually become state law. "We are becoming a surrogate for the U.S. in stem cell research," says institute president Zach Hall. Likewise, the National Academies has announced that it will form a permanent panel to offer up-to-date guidance to stem cell researchers. Funding from private sources will help it apply guidelines proposed in an April 2005 report.

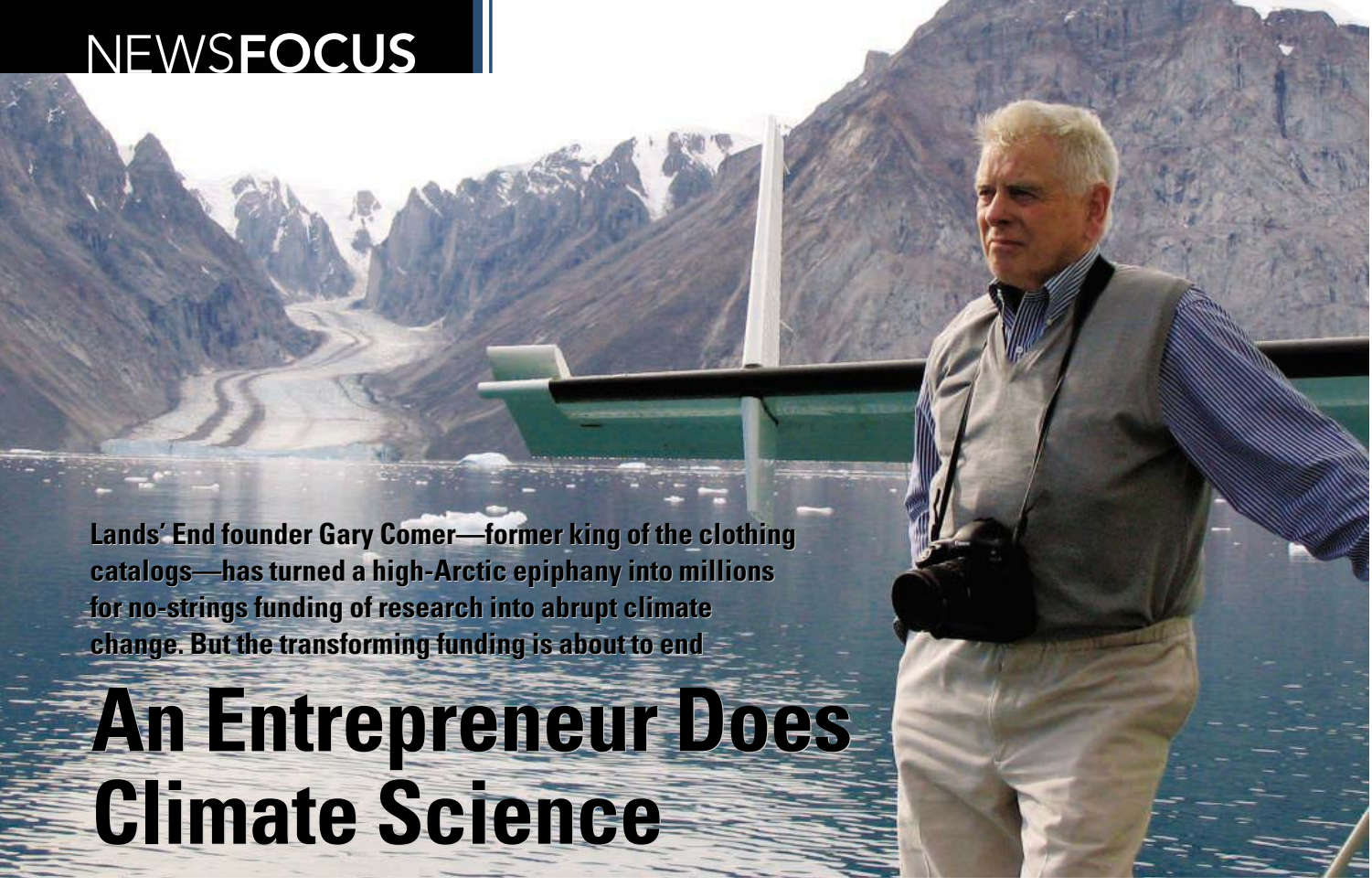
—CONSTANCE HOLDEN

Gene Grant Funds Less Chaff, More Wheat

The U.S. Department of Agriculture is betting on a new high-throughput, molecular-based breeding program to help it win a 7-year battle against a devastating rust plague. Last week, a consortium of 20 university and government labs received $5 million to pinpoint genes that provide resistance to the rust. Researchers will find genes using known markers, or landmarks, on wheat chromosomes near these genes.

Starting with a few dozen of these markers, scientists hope to identify tens of thousands of them over the next 4 years, says project director Jorge Dubcovsky of the University of California, Davis. The markers should accelerate the development of strains with multiple disease-resistant genes, higher gluten content, and increased yield. "The sooner we can solve the disease issue, the better for the growers," says Bonnie Fernandez, executive director of the California Wheat Commission in Woodland. Dubcovsky says the first strains may on sale by 2008.

—ELIZABETH PENNISI



Lands' End founder Gary Comer—former king of the clothing catalogs—has turned a high-Arctic epiphany into millions for no-strings funding of research into abrupt climate change. But the transforming funding is about to end

An Entrepreneur Does Climate Science

GARY COMER KNEW SOMETHING WASN'T right. John Franklin and 128 companions had famously tackled the Northwest Passage in 1845, and none of them returned. Roald Amundsen finally conquered the passage in 1906; it took him 3 years. Yet in the summer of 2001, Comer was motoring unscathed through open Arctic waters that should have been ice-clogged. He made the transit over the top of North America in just 19 days. "We were able to do it, and so many people had failed," he says. "Something had happened."

It was global warming, Comer decided. Months later, he began to work on the problem of sudden changes in his beloved Arctic. "I had some cash," he recalls, having the day before cleared about $1 billion selling his Lands' End catalog business. And his sense of urgency had been sharpened by a recent diagnosis of prostate cancer. So he told a Nobel-laureate geochemist, "I'd like to do something that would be helpful" about global warming.

Thus began Comer's freewheeling research enterprise targeting climate's propensity for sudden, potentially debilitating shifts. He hoped to awaken the American public to the threat of global warming. His approach was unconventional but not so surprising coming from a world-class sailor, empire builder, and former ad man: Identify a few top-notch senior scientists; give them money, unsolicited, to support up-and-coming young scientists; fund fieldwork nobody else would touch; and then—less predictably—jump in and enjoy the science.

Tens of millions of dollars later, Comer has made an impression. "He changed the field" of abrupt climate change, says glacial geologist George Denton of the University of Maine, Orono. And "he changed my life. He's something very special. This guy is thinking about the world; he thinks something has to be done." Comer hopes that money well spent on a key climate unknown will prompt the federal government to take up the burden. "Who needs to go to the moon?" he asks. "Take care of Earth."

From dinghy to deep sea

Comer's entrepreneurial career as well as his foray into science funding really began on Lake Michigan. Born to a working-class family and raised on the South Side of Chicago, he began sailing small boats off Chicago at age 14. By age 30, Comer had sailed his 7-meter Star Class *Turmoil* to second place in the world championships. At the same time, he was having second thoughts about his 10-year advertising career as a copywriter at Young & Rubicam, a job he had approached through sailing friends. So he started a sailing-gear supply company, Lands' End Yacht Stores (misplacing the apostrophe by typo), which morphed into the huge catalog and Web apparel business of Lands' End Inc.

The *Turmoil* boats grew as well, and lost their sails, until Comer was motoring to remote coasts in a 46-meter *Turmoil* that "from the outside looks like a fishing vessel," as one guest puts it, "and from the inside like The Four Seasons." On it, he traveled more than a quarter-million kilometers, much of it to high latitudes. "My lifelong fascination with the Arctic and things Arctic started [when] I became obsessed with news of the plane crash that took the lives of pilot Wiley Post and humorist Will Rogers" near Barrow, Alaska, he wrote in a journal. "I was 10. … It was the beginning of my fascination with airplanes, pilots, Eskimos, igloos, and life in the bitter cold. … The sheer strangeness of it all—I was amazed."

Far traveler. Comer's *Turmoil* has carried scientists to Greenland's glaciers and beyond. [illegible]

CREDITS (TOP TO BOTTOM): RICHARD ALLEY; G. COMER

Climate moneyman. Gary Comer funds and transports scientists who study abrupt climate change in the Arctic.

Charles Hollister, a deep-sea sedimentologist, was the first to begin turning Comer's adventurous spirit toward science. By the late 1990s, Hollister, a longtime Woods Hole Oceanographic Institution (WHOI) researcher, had become an administrator and fundraiser there. What better person to interest in oceanography than this well-heeled adventurer of the sea? Hollister contacted Comer and got an invitation to cruise the Kurile Islands northeast of Japan with Comer on *Turmoil*. Hollister died in 1999 in a fall while hiking, but the new WHOI director of development, Daniel Stuermer, soon invited Comer on a different sort of ocean expedition: heading down in the deep submersible *Alvin* to the subsea mountain range of the East Pacific Rise.

The tipping point

"Gary got excited," says Stuermer. But Comer had not yet made up his mind to spend major amounts of money on anybody's science. That came after his "over-the-top cruise." On returning from the Northwest Passage, he called Stuermer. "I'm really worried," Stuermer recalls him saying. "I shouldn't have been able to do that. Global warming is really a problem for the world. What are we going to do about it?" That began Comer's career in funding climate change research.

"There wasn't any plan," Comer concedes. Instead, he picked up "little threads" that presented themselves. There was, however, a new motivation. In December 2001, he learned he had advanced prostate cancer. That "made me realize whatever I was going to do, it was time to do it," he says. And "it's important to let other people know there are things you can do with money that are very satisfying and helpful."

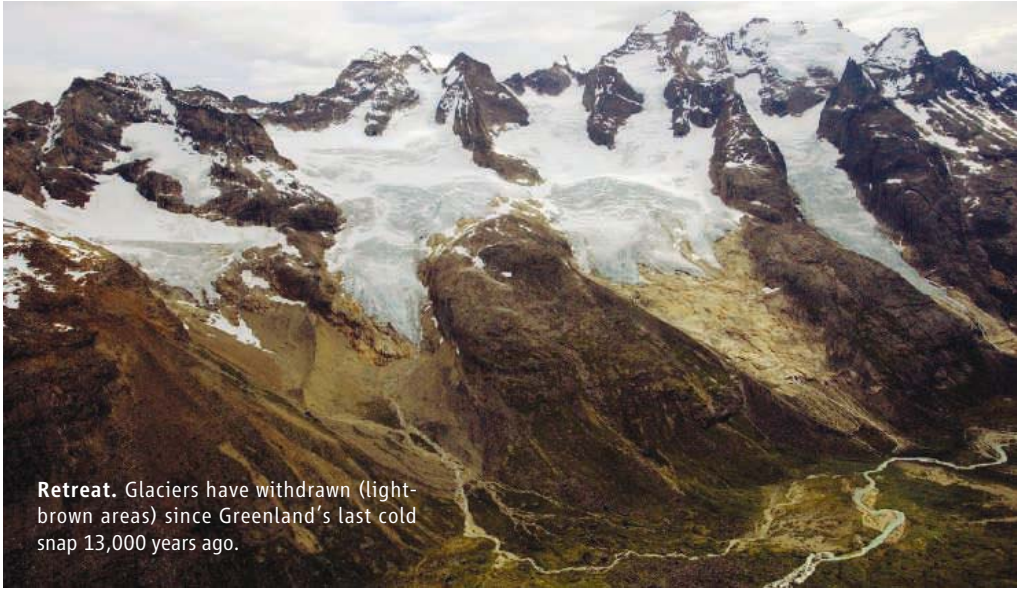
One thread came in conversation with a Chicago friend in early 2002. When global warming came up, the friend mentioned a scientist—the friend's ex-wife's cousin's husband—who would share Comer's interests. So Comer went to visit the laboratory of F. Sherwood Rowland, an atmospheric chemist at the University of California, Irvine, who had won the 1995 Nobel Prize for his role in pinning ozone losses on chlorofluorocarbons and was now studying methane, a powerful greenhouse gas.

That summer, Comer sent his jet to pick up Rowland and his wife near Irvine. They were to meet him on *Turmoil* in Victoria, British Columbia. Comer arrived late but exuberant. He had just sold Lands' End to Sears for $1.9 billion, clearing about $1 billion cash on the deal. So he popped the question: "If I wanted to put $1 million into climate change," Rowland recalls him saying, "what should I do?"

Rowland had a ready answer that set the core structure of Comer's funding program: Comer should support 10 graduate and postdoctoral fellowships at $50,000 per year for 2 years. Rowland offered to take one fellow and choose researchers to handle the rest. Comer liked the idea, but he thought it called for "not enough money, too many people." Instead, he proposed five fellowships at $100,000 per year to run for 3 years—overhead-free, he would insist.

On to abruptness

Comer wasn't finished. He had "started out wanting to bring the climate-change problem to public attention," he says. He intended to be in the thick of climate research. And for that, it seemed, he needed geochemist Wallace Broecker. Comer kept coming across Broecker's name, whether from Stuermer, an environmentally connected friend, or his own reading. A longtime researcher at Lamont-Doherty Earth Observatory in Palisades, New York, Broecker was obviously the point man on nasty surprises that might be lurking in the looming greenhouse (*Science*, 10 July 1998, p. 156). Comer wrote Broecker a letter about his disturbing trip through the Northwest Passage, but Broecker was too busy teaching near the end of the semester to go see Comer at his homes in Waukesha, Wisconsin, or Chicago. So Comer came to Broecker.



Retreat. Glaciers have withdrawn (light-brown areas) since Greenland's last cold snap 13,000 years ago.

Within a few minutes of meeting Broecker in his hotel's coffee shop, Comer popped his question again: "Wally, I want to help you," Broecker recalls him saying. "What can I do for you?" Rowland's fellowship idea sounded good to Broecker, especially with a focus on abrupt climate change. This was the climate system's big unknown, Broecker argued. Sudden shifts in climate had rattled the hemisphere if not the globe not so long ago, and the growing greenhouse could conceivably trigger a recurrence. Broecker was worried that [illegible] ocean conveyor that warms the far northern Atlantic. If the greenhouse shut it down, as something did repeatedly more than 10,000 years ago, there could be hell to pay.

"I became pretty tight with Wally," Comer says. "I've always had an interest in science, though it was nothing I studied in school. Wally was a great inspiration; he has a knack for explaining things. He came up with really interesting things to do. His interests became my interests." Broecker returns the compliments. "He's really made a difference to me," he says. "It's been much, much more than the money. He caught me at a time when I was thinking of retiring. He inspired me and gave me a mission."

The Comer way

Once he made his initial contacts with the scientific community, Comer grew his funding much as he grew his business. He rooted out good people and let them loose, while keeping a close eye on how they did. "He's very straightforward, very direct," says Stuermer. "If you're satisfying him, you know. If not, you know that." Stuermer's marching orders were simple: "Do things that are important but won't be done by government," he recalls. "Choose people Comer would like—that is, respect and admire." And finally, Comer said, "Dan, I'm letting you guide me here; don't [mess] up."

No one has messed up so far. Comer initially gave $1 million to WHOI's Climate Institute, followed by an unrestricted $5 million gift to WHOI, some of which went to climate-related research. He expanded his centerpiece, the Comer Fellows, to 31 "mentors" running two fellows each over 5 years. The fellows program will end 2 years from now, if all the pending renewals go through as expected, for a total of about $6 million. Most of the mentors were chosen by Rowland and Broecker and some

CREDIT: G. COMER

more after Broecker brought in glaciologist Richard Alley of Pennsylvania State University in State College to form a troika of overseers. "It's quick funds," says Comer. "We don't have a peer-review system." His motto: "Keep it simple." In addition, Comer has set aside $5 million to be distributed with advice from the troika. Unsolicited proposals are not considered.

Comer has also picked up the annual tab of about $50,000 to support the "Changelings," a small group of abrupt-climate-change specialists who periodically gather with invited experts to ponder special problems. After starting the Changelings in the mid-1990s, the National Oceanic and Atmospheric Administration (NOAA) dropped the funding in a cost-cutting move. And Comer is covering $18 million of the $40 million needed to replace Lamont's 50-year-old "Quonset hut" of a geochemistry building, where Broecker has spent all 53 years of his career. The move is reminiscent of Comer's 2001 $21 million contribution to help build a children's hospital for the University of Chicago. Indeed, his climate contributions are in much the same spirit as the several million he has contributed over a few years to stabilize schools and the community in his childhood inner-city neighborhood of Chicago.

Field workers. Left to right, glacial geologist Denton, glaciologist Alley, environmental organizer Phillip Conkling, funder Comer, and geochemist Broecker tackled the icy wastes of Greenland.

Big fish, smallish pond

Millions may be small potatoes in biomedicine, but in a subspecialty of climate change, it's real money. The pace of Comer's spending on research over 6 years will equal or exceed that of NOAA funding specified for abrupt climate change. And that's about the only U.S. public funding directed toward that area. Comer's contribution is "a very large and beneficial infusion," says Alley. "There's an immense amount of really good science. The total output of the field is much greater than it would have been otherwise."

Six million dollars' worth of cheap, productive postgraduate labor is in fact buying a good deal of science. For example, one of the first people Rowland contacted was geochemist Jeffrey Severinghaus of the Scripps Institution of Oceanography in San Diego, California. The call came out of the blue: "This is not a contest. You've already won." He'd won two fellows, no strings attached. "Gary clearly has an interest in abrupt climate change," says Severinghaus, "but there's been no heavy-handed direction."

One of Severinghaus's fellows showed that carbon dioxide was not the ultimate driver of the last deglaciation; that work was published in *Science* in 2003. A second fellow refined Severinghaus's geochemical "thermometer," which used air trapped in ice cores to document Greenland's stunningly abrupt 10°C temperature drops during the last ice age.

"It's a very effective way of funding science," says climate modeler Stefan Rahmstorf of the Potsdam Institute for Climate Impact Research in Germany, another mentor. At first, his offer from Rowland "read like a Nigerian e-mail scam," he says. He found it "wonderful to be able to think freely, … follow scientific instincts, and explore things" without all the usual bureaucracy.

Comer has also taken researchers on four field trips to high latitudes. Two expeditions were to survey areas of Canada, in part using Comer's 12-seat jet, eight-seat Caravan prop plane, and a chartered helicopter. There researchers—including Comer, Broecker, and Denton—found signs that the trigger for an abrupt cooling 13,000 years ago called the Younger Dryas may not have been a gush of glacial meltwater, as many had thought, because the meltwater was still blocked by ice then.

Two other field trips took *Turmoil* to southern Greenland and into Scoresby Sund on the east-central coast to obtain the glaciological

of the Younger Dryas. Working off of *Turmoil* and reconnoitering in Comer's float plane or helicopter, Denton, Alley, and others studied the ridges of debris deposited by glaciers at their maximum extent, when summers were coldest. Drawing on that fieldwork, Denton, Alley, Comer, and Broecker reported last year that a broad expanse of North Atlantic ice cover seems to have been key to a brutally cold Younger Dryas. That implies that in a future greenhouse world—when sea ice is diminished, not expanded—a repeat cooling like the Younger Dryas would be less likely.

As evidenced by his prominent authorship on the resulting papers, it was these field trips that drew Comer deeply into the science. The authorships were "not an honorary thing," says Alley. "He was in the discussions, he was contributing." That's the way he's always been, says his daughter Stephanie Comer. "He's someone who barely made it out of high school and never went to college," she says. "But he figured out how to educate himself. He'd find the best people out there who knew about, say, inventory control, and he'd learn through them. He approaches everything that way." Her father's initial hope of bringing in the general public proved unrealistic, he says. Instead, "I became interested in the science side, understanding it myself."

Good but not forever

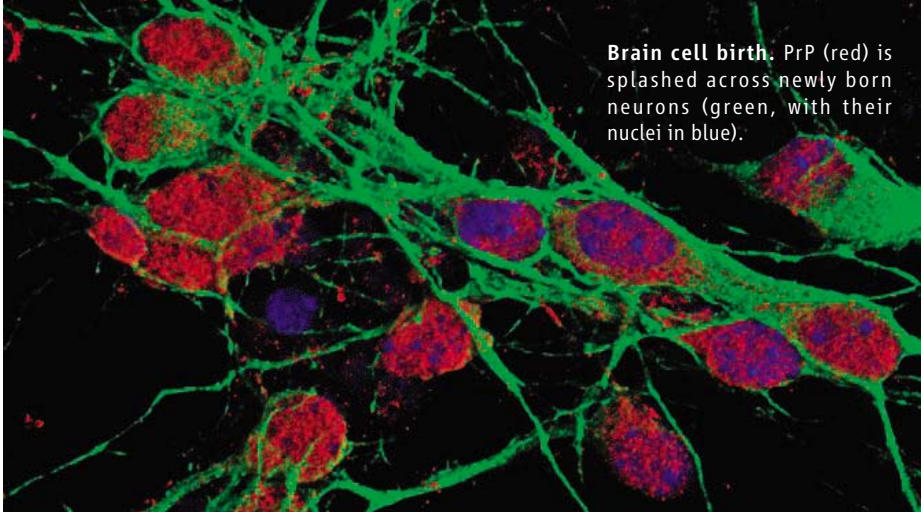
Whatever the motivation, the Comer approach has been well received in the broader community. "They're good people doing good science, no doubt about that," says paleoclimatologist Thomas Crowley of Duke University in Durham, North Carolina, who has received no Comer support. El Niño modeler Mark Cane of Lamont, who only recently got "a little money" from Comer, says, "A lot of good work has come out of it. Climate research in general is not very well funded these days, so he's keeping areas alive that would be in serious trouble." That's okay with non-Comer recipients such as Crowley; it's Comer's money, not the public's, and he seems to know what to do with it.

Well received or not, Comer's program is not open-ended. When fellowship extensions end in 2 years, "I'm out of funds for it," says Comer. "We're trying to get things started, things that wouldn't be supported otherwise. [After that], Uncle Sam is going to have to take over. The fellowships enabled a group of 60 or 70 people to find jobs in climate research, particularly abrupt climate change. That was the purpose."

Comer does have one other iron in the climate fire. Backing up the science he's funded, he is sinking millions a year into a small Arizona company developing a method for extracting the main greenhouse gas—carbon dioxide—right out of the air for permanent storage underground. If some new science can't win the day, perhaps some innovative engineering can.

–RICHARD A. KERR

CREDIT: PROVIDED BY P. CONKLING



Brain cell birth. PrP (red) is splashed across newly born neurons (green, with their nuclei in blue).

CELL BIOLOGY

The Prion Protein Has a Good Side? You Bet

Prion diseases are caused when a normal but enigmatic protein misfolds and turns deadly. New work is beginning to unravel what this protein does in the first place

Why has the human body preserved a protein that can turn deadly? This mystery has bedeviled the field of prion diseases such as "mad cow disease," in which a normal protein, called PrP, misfolds and assaults brain tissue. Prion diseases are exceedingly rare, but PrP is not. It's found throughout the body, from the blood to the brain.

Now two papers in the online *Proceedings of the National Academy of Sciences*, one published 7 February and the other scheduled to appear this week, may offer the beginnings of an explanation. The research found that PrP is expressed on the surface of stem cells in bone marrow and on cells that become neurons. In both, PrP seems to offer a guiding hand in cell maturation.

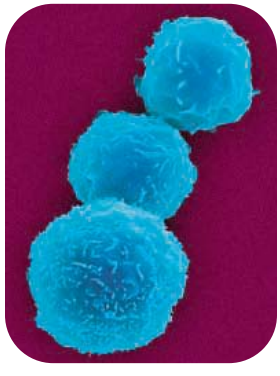
What this means, exactly, and how it's occurring haven't been deciphered. Doing so might shed light on what sends PrP morphing into prions. But to scientists accustomed to the PrP black box, even nuggets of news are welcome. Says Neil Cashman, a neuroscientist at the University of British Columbia in Vancouver, Canada, and scientific director of PrioNet Canada: "It's clear beyond a shadow of a doubt that they've established a function" for the enigmatic protein.

The project began in the lab of Harvey Lodish, a stem cell biologist at the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts. There, lab members were struggling with how to keep their mouse stem cells dividing in petri dishes. Postdoc Cheng Cheng Zhang noticed PrP on the surface of cells that facilitated the division of hematopoietic stem cells, which go on to form the blood and immune system. PrP was also "hugely expressed" on the surface of these stem cells, says Lodish.

Intrigued, Zhang and Lodish teamed up with Whitehead prion expert Susan Lindquist and graduate student Andrew Steele. Mice without PrP, they found, had healthy blood systems. But although that might suggest PrP played no role, the scientists knew that when one molecule becomes defective, others may make up the deficit and keep the organism healthy. They wondered whether that might be the case here, with some proteins filling in for PrP's absence to keep the blood system humming. The scientists decided to stress the animals' hematopoietic stem cell system to better see whether PrP had a role in its development.

The group irradiated the mice to kill their bone marrow and then performed a series of hematopoietic stem cell transplants. The irradiated mice were infused with a mix of hematopoietic stem cells, half from mice expressing PrP and half from mice that didn't. After a few months, these animals became donors for a new set of irradiated mice. The question was whether the stem cells lacking PrP repopulated the blood system as readily as those with the protein did. If PrP and non-PrP cells were equal, half of the animals' blood cells would be expected to boast a surface marker showing they came from PrP-positive cells, and half wouldn't.

But by the third transplant, roughly 71% of circulating blood cells had the surface marker, suggesting that stem cells with PrP flourished more readily than those without. As further evidence, the researchers used a retrovirus to reinsert PrP into hematopoietic stem cells; this restored their ability to thrive.



In the blood. Hematopoietic stem cells, which form the blood system, carry the prion protein on their surface.

Although PrP is clearly "mediating survival" here, says Gerald Spangrude, a stem cell biologist at the University of Utah in Salt Lake City, "is [the protein's role] transient, or is PrP needed throughout the life of the stem cells?"

Another question is whether PrP expression alone explains the results. The mouse strains used have slight genetic differences beyond PrP expression, says viral immunologist Bruce Chesebro of the Rocky Mountain Laboratories in Hamilton, Montana, and these "contaminating background genes" might affect the results.

Recalling a lecture on early nervous system cells by Harvard University neuroscientist Jeffrey Macklis, Steele began to wonder whether PrP had a hand in primitive neural cells as well. Macklis's postdoc Jason Emsley agreed to help Steele and Lindquist find out.

First, the group examined whether primitive central nervous system cells in normal mice expressed PrP. The protein's expression grew more apparent as early neural cells developed. PrP was also expressed in "whopping amounts" in mature neurons, says Steele—something others had previously found. But it was undetectable in other types of brain cells, an observation that's both supported and contradicted by earlier work. In both embryonic and adult mice, PrP was expressed at brain locations "right where neurons are born," says Macklis.

Further findings, in tissue culled from mice genetically engineered to overexpress PrP and mice that lacked the protein, suggested that PrP might help guide the decision of a neural progenitor cell to become a neuron. In petri dishes, 26% of neural progenitor cells from PrP overexpressors became neurons, compared with 18% from normal mice and 14% from PrP knockouts. But these and other researchers haven't found differences in the brains of adult animals with and without PrP, something that's sown confusion about PrP's healthy function. Rather than implying that PrP is governing neural differentiation, says Macklis, the work suggests that "it's another fine-tuning knob, one of many fine-tuning knobs on the amplifier."

Now, the team hopes to do what was done for hematopoietic stem cells: Challenge the system as a way to strip bare PrP's role. Rather than receive stem cell transplants, the mice are being placed in mentally stimulating environments, which should trigger growth of new neurons. Instead of barren cages, they're housed with exercise wheels, cotton for nest-building, and hidden granola treats, says Steele. Will mice without PrP grow fewer neurons? That's the next question on the docket.

–JENNIFER COUZIN

CREDITS (TOP TO BOTTOM): HANDE OZDINLER; DENNIS KUNKEL MICROSCOPY INC.

Is the Education Directorate Headed for a Failing Grade?

Education researchers say that a sinking budget, a leadership vacuum, and an administrative reshuffle put NSF's education activities at great risk

Speaking this month at an elite science high school in Dallas, Texas, President George W. Bush told the assembled students that the United States "needs a workforce strong in engineering and science and physics" to remain the world's top economic power. His words would seem to bode well for precollege activities funded by the National Science Foundation (NSF), the only federal agency with an explicit mission to improve science and math education. But 3 days later, the president unveiled a 2007 budget request that would cut—for the third straight year—a 4-year-old program at NSF aimed at doing exactly that.

The decline of the Math and Science Partnerships (MSP) program, which links university science and math faculty with their local elementary and secondary schools, is only one of many problems facing NSF's $800 million Education and Human Resources directorate (EHR). The directorate's once-robust budget, which includes efforts to foster greater participation in the sciences by women and minority groups, has turned anemic at the same time the president has proposed a $380 million initiative to improve elementary and secondary school math and science funded by the Department of Education (ED) (*Science*, 10 February, p. 762). EHR has been run for more than a year by a temporary head after its top official, Judith Ramaley, was denied an opportunity to stay on. (She is now president of Winona State University in Minnesota.) And this month its acting director, Donald Thompson, quietly announced plans for a major internal reshuffling that is being seen as accelerating a move away from direct intervention in the classroom. Several researchers worry that the change will reduce the impact of NSF's strong research-based approach to educational reform and substitute lower-quality programs run by ED.

Together, these developments have caused "a dangerous downturn" at EHR, says Gerry Wheeler, executive director of the National Science Teachers Association in Arlington, Virginia. Many education researchers say they fear that bleak picture may scare away any outside scientist or science educator looking to make an impact in Washington, D.C. "Maybe there's some brave soul" who might take the job, says Manuel Gomez, vice president for research at the University of Puerto Rico. "But if you put enough constraints on the problem, then it doesn't have a solution."

A theoretical physicist and science educator, Gomez has more than a passing interest in what happens to the directorate. *Science* has learned that Yerodin Proudly Presents, That for Support EHR on a list compiled by an outside search committee in late 2004 and one of three people interviewed by NSF officials for the post last March. The others were Margaret (Midge) Cozzens, a mathematician and former EHR division director now at the Colorado Institute of Technology in Broomfield, and Claudia Mitchell-Kernan, head of graduate studies at the University of California (UC), Los Angeles, an anthropologist and former member of NSF's oversight body, the National Science Board. All confirm they met with NSF Director Arden Bement to discuss the post, and all say that the prospects for the directorate gave them pause.

Gomez echoes many in the field when he complains that the directorate's budget has dwindled at the same time "everybody recognizes that strengthening human resources and education are essential for the health of U.S. science and technology." In the last 2 years, EHR's budget has fallen from $939 million to $796 million—and that's after Congress added nearly $60 million to a Bush Administration request for only $737 million. Next year's request is for $816 million, a 2.5% rise that trails the overall 7.9% increase sought for the foundation as a whole. "If the EHR budget stays flat, there's no hope of accomplishing what corporate America says is needed to improve the U.S. workforce," says Wheeler, a member of the search committee. "I think we're facing a real crisis." Notes Mitchell-Kernan, "I don't know why anybody would go there just to mind the store."

Researchers and policymakers are especially troubled by the cuts to NSF's precollege programs, part of what they see as a conscious shift of resources by the Bush Administration to ED. In addition to hosting the proposed math and science education initiatives in the president's 2007 budget, ED runs its own version of the MSP program, which has grown from $12.5 million in 2002 to $182 million this year. In contrast, the MSP program at NSF would receive $47 million, down from $63 million this year and $140 million in 2004.

That shift in the MSP program is a mistake, say researchers and policymakers, who believe that it will deprive U.S. schools of effective, science-based improvements in teaching and learning. "Both programs should be continued because they serve different purposes," says Representative Vernon Ehlers (R–MI), a former physics professor and indefatigable campaigner for improving U.S. science education. "The ED money goes to states as block grants," he explains, and each state doles out the money as it sees fit. In contrast, he says, NSF's "are peer-reviewed grants for individual projects" that survive a rigorous competition. "It's the difference between a meritocracy and an egalitarian system," says Representative Bob Inglis (R–SC), who chairs the panel's research subcommittee. "NSF looks for the best, while ED is supposed to serve everyone."

A reeducation. NSF Director Arden Bement (*left*) has given acting EHR Director Donald Thompson the green light to reshape the education directorate.

CREDITS (LEFT TO RIGHT): CHRIS MADDALONI; OLPA/NSF

Bement and his senior education staff say that the critics are wrong and that the outlook for the directorate is actually very bright. For starters, Bement says he didn't offer the job to any of the selection committee's choices because Thompson "is better than any of the people that I interviewed." And although Bement told *Science* he is still weighing "two or three candidates," he says he has total confidence in Thompson's ability to move EHR forward.

Distancing himself from those who call for more money, Bement says the president's budget request "allows us to address our priorities" within EHR and to grow several important programs. Efforts to broaden participation in science by minorities and women, he notes, would jump by 21%, to $144 million. The internal realignment is another sign of good health, Bement says: "We've had a number of fragmented programs that overlap, and these changes will allow us to give them more resources."

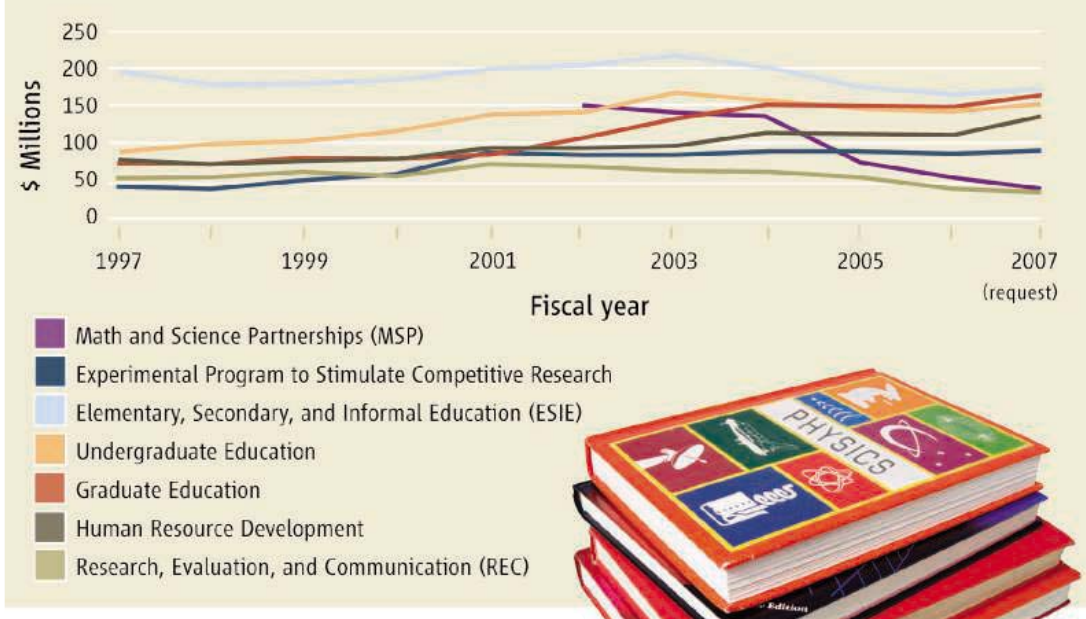
Bement, who was confirmed in November 2004 after 9 months as acting director, signaled his plans to move the foundation away from direct intervention in the classroom when he told a House spending panel last year that "we know what works" and that many of EHR's programs "are in the flat part of the learning curve." Last week, he went further, telling the House Science Committee that the large-scale, systemic initiatives NSF funded in the 1990s at the state and local level were "test beds" to demonstrate the value of good practice and strong involvement by local industry and the community. "These lessons have been learned," he says, "and now the time has come to propagate that message to the nation's 15,000 school districts."

The realignment reflects that shift in emphasis. The Division of Elementary, Secondary, and Informal Education within EHR has morphed into the Division of Research on Learning. And its major program components, which once included words such as "teaching" and "instructional materials," have been amalgamated under the rubric "Discovery Research: K–12." Those changes, says Bement, will give program officers greater flexibility "to address some grand educational challenges, such as finding new ways to make science exciting for elementary school children by incorporating some of the recent advances in the field." Thompson, a former professor of urban studies and education school dean, says that the realignment will also put "research and evaluation closer to the K–12 programs" and ensure that every program solicitation and proposal submitted to NSF addresses "not just the goals of discovery but also how the results will impact learning in the classroom."

But figuring out what works is an enormous challenge for evaluators. For example, a 2004 report by the National Research Council (NRC) of the National Academies (*Science*, 11 June 2004, p. 1583) examining nearly 700 studies of 13 math curricula developed by NSF found that only 21% were rigorous enough to be evaluated. And the committee could find none that clearly demonstrated effectiveness. "I don't think that [NSF and ED] have managed to find much of substance" in their evaluation of these curricula, says Jere Confrey, a math educator at Washington University in St. Louis, Missouri, and chair of the NRC panel. "So it's not clear to me how they expect to build on existing work."

Bruce Alberts, who pushed hard for reform of K–12 science and math education during a recently concluded 12-year stint as president of the National Academy of Sciences, is even blunter about the problem. "It's not enough to ask if a particular program is working," says Alberts, now back at UC San Francisco. "What you want is general information that would help people do it better the next time." Alberts doesn't spare NSF in his criticism of what evaluation research has contributed to student performance. "I don't think NSF evaluations have been very effective in providing anything that is useful to anybody else," he says. "Maybe NSF education programs needs to proudly present, Thx for Support

Follow the Money



Ebb and flow. Precollege programs have borne the brunt of the recent funding cuts within the directorate. (REC and ESIE have been combined starting in FY 2007, and the MSP program is joining undergraduate education.)

Part of the obstacle to reforming EHR is recognizing its limitations. NSF's budget isn't big enough, nor does it have the direct links to school districts, to improve U.S. science and math education. Its strength lies in working with the academic community, and the challenge for NSF officials is to meld that expertise with the practitioner-driven focus of ED. "All the time I was in D.C., there has been a war between ED and NSF over jurisdiction on K–12 programs," says Alberts. "If I were Arden, I'd try to straighten out [the relationship] with ED before I went ahead [with any changes] at NSF."

Bement says that's exactly what he's been doing, citing ongoing meetings with top ED officials to coordinate joint activities. Education Secretary Margaret Spellings says she hopes NSF can play a role in two math initiatives for elementary and middle school students proposed for 2007, although NSF has not been given any funding for either initiative.

Within NSF, Thompson says he's doing similar outreach across the foundation's six research directorates, teaming up with them on education programs in fields such as biology, geology, nanotechnology, and the International Polar Year that runs through 2007. And members of his staff say those efforts are working. "We're trying to increase the research component of our programs by working with the other directorates," says John Cherniavsky, a senior EHR adviser within the new Division of Research on Learning. "Nobody likes budget cuts, but you learn to make the best of them." **–JEFFREY MERVIS**

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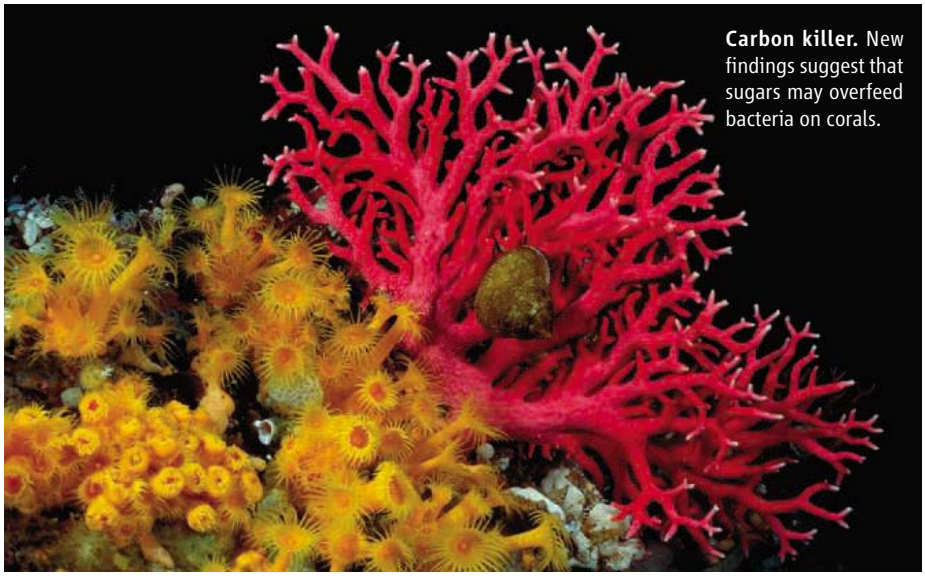


Carbon killer. New findings suggest that sugars may overfeed bacteria on corals.

Don't Sugarcoat Corals

Potentially shaking basic assumptions of marine biologists, the first large-scale ecotoxicity study of coral has identified a new and surprising suspect for what may be killing reefs worldwide: organic carbon in the form of simple sugar molecules.

Coral reefs are under global assault; Caribbean reefs, for example, have lost 80% of their coral cover in the last 3 decades. As coastal populations near reefs have skyrocketed, scientists have fingered phosphates, nitrates, and ammonia as the most likely culprits. They surmised that these pollutants aid the growth of algae that compete with coral for space. The new results, presented at the annual meeting of AAAS (the publisher of *Science*), suggest that carbon-induced bacterial growth may also be a major problem.

In 2003, marine biologist David Kline of the Smithsonian Tropical Research Institute in Balboa, Panama, performed more than 3000 individual monthlong experiments on coral heads sampled from the Panamanian coast. After dosing the corals with solutions of basic chemicals found in sewage and agricultural runoff, he found that, on average, almost 35% of corals exposed to carbon compounds died compared to about 7% of those given nitrate or phosphate.

Separate experiments showed that sugars led to an explosive growth of coral-associated bacteria not caused by other chemicals. If this holds true in the ocean, says Kline, corals already under stress from warmer water temperatures and the loss of fish and urchins that eat algae may succumb directly to the rapid growth of the normally symbiotic bacteria. Or they may be weakened enough that the fleshy algae finally win out. "Carbon-loading disrupts the balance between coral and its associated bacteria, leading to disease," says Kline, who will detail the work in *Marine Ecology Progress Series* next month.

"Retrospectively, it makes sense that the bacteria would benefit from the sugars, but it's not something I would have predicted," says Mary Alice Coffroth, a coral reef biologist at the University at Buffalo, New York. "Sugar has never been looked at like this."

Noting that 7% to 8% of the coral controls in the study died, coral biologist Alina Szmant of the University of North Carolina, Wilmington, cautions that Kline's experimental system may lack the natural water flow that corals use to fight bacteria. The microbes may have an artificial advantage, she says. Forest Rohwer of San Diego State University in California, a co-author of the upcoming paper, counters that most corals survived, indicating that the system was robust.

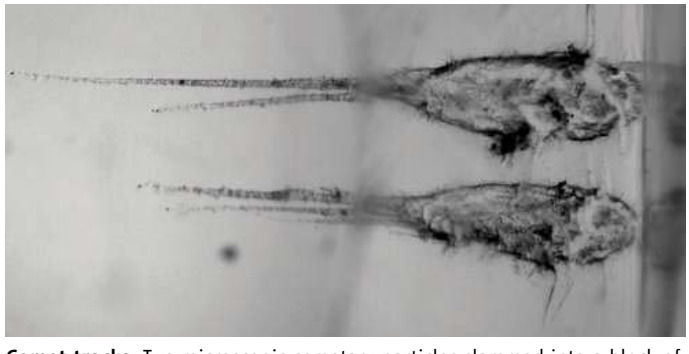
Kline says these new results should motivate coastal officials to utilize basic sewage treatment that would reduce organic carbon levels—only 10% of sewage flowing into the Caribbean is treated—and check organic carbon levels along with other pollutants. "We are not monitoring a critical component of the water in the system," he says. **–ELI KINTISCH**

A First Look at a Comet's Dust

The success of NASA's Stardust mission, which returned material from comet Wild-2, is giving planetary scientists a chance to test theories about the composition and formation of the early solar system. "We're seeing a variety of things we absolutely know came from a comet," says Stardust principal investigator Donald Brownlee of the University of Washington, Seattle, who in St. Louis described the first analyses of microscopic cometary particles trapped in the craft's 132 ice-cube-sized blocks of aerogel.

So far, scientists have identified glassy and crystalline compounds, including iron sulfides. Although sulfides were not unexpected, there had been no spectral evidence that comets contain sulfur, notes Brownlee. Mission scientists have also found hints of organic matter and so-called GEMS (glass embedded with metal and sulfides); the latter are thought to be from stars.

Comets originated at the periphery of the rotating cloud of gas and dust that formed our solar system and likely retain pristine material from that time. After Stardust's 4.6-billion-kilometer, 7-year roundtrip, we now "have exciting samples from the edge of the solar system 4 billion years ago," says Brownlee. Mission scientists [illegible] particles at the ends of impact tracks (above) preserved in six aerogel cubes and expect to present much more data at a meeting next month. "I've made all sorts of predictions [about the early solar system], and I figure half of them will be wrong," says Joseph Nuth of NASA Goddard Space Flight Center in Greenbelt, Maryland, with a smile. **–JOHN TRAVIS**



Comet tracks. Two microscopic cometary particles slammed into a block of aerogel, fragmenting and making these tracks.

Hot Times for the Cretaceous Oceans

Ancient evidence from the sea floor suggests that the ocean surface some 90 million years ago was hotter than the water in a hot tub—and that climate modelers are underestimating the link between carbon dioxide and warming.

The new data, presented in St. Louis, Missouri, by climate modeler Karen Bice of Woods Hole Oceanographic Institution in Massachusetts, comes from three sea-floor cores drilled in 2003 off Suriname in the tropical Atlantic. The cores contain rocks from the Cretaceous period, 65 million to 145 million years ago. By studying microscopic shells in shale and analyzing oxygen isotopes and trace elements, the researchers concluded that sea surface temperatures then may have reached 42°C—14° warmer than the tropical Atlantic is now and 5° higher than previous estimates for the period. Studies of organic material from the cores confirmed previous estimates that atmospheric carbon dioxide levels during 20 million years of the Cretaceous were between two and six times the current level of 380 parts per million.

A report on the research will be published soon in *Paleoceanography*, but it's already making waves. The proposed blistering ocean temperatures bring "into question whether there are any limits to the temperature in the tropics in the future," says paleoclimate scientist Mark Chandler of Columbia University.

The paleoclimate results may also retune the computer climate models currently used to forecast future global warming. Running GENESIS, a top atmospheric model developed at Pennsylvania State University in State College, Bice had trouble getting the carbon levels she had measured in the cores to produce the high temperatures she had obtained. To make the model match her temperature estimates, Bice had to assume that another greenhouse gas, methane, was 30 times as abundant in the Cretaceous atmosphere as it is today. Most scientists consider that figure far-fetched, although they don't yet have a way to estimate the actual Cretaceous level. Bice concludes that climate models predict too little warming as carbon dioxide skyrockets.

Drilling down. Karen Bice analyzes sea-floor cores for clues about ancient climate.

Not everyone accepts those results. "The fascinating and alarming aspects of the study are probably simply not true," says paleomodeler Matthew Huber of Purdue University in West Lafayette, Indiana, arguing that Cretaceous plants could not have survived such high temperatures. But Bice says Cretaceous organisms had time to evolve adaptations to the warming climate.

Bice notes that the Intergovernmental Panel on Climate Change is asking modelers to predict how temperatures would be affected by a fourfold increase in carbon dioxide—roughly what the ocean core data suggest existed during the Cretaceous period. Other leading models share GENESIS's low sensitivity to greenhouse gases, Bice says, so if her results hold up, global warming "is going to be more dramatic than what is shown in these models."

–ELI KINTISCH

Preyed Upon, Hominids Began to Cooperate

Watch the 6 o'clock news or stroll through a natural history museum of spear-wielding cavemen, and you might think humans have been killers since the dawn of time. But our hominid ancestors actually lived as prey, a researcher argued at the meeting. And our past as prey, he speculates, laid the foundations of society by forcing those ancestors to live together peacefully in groups.

Dogma has it that humans and our ancestors have always been violent and warlike, in part because we evolved as hunters, says biological anthropologist Robert Sussman of Washington University in St. Louis, Missouri. Proponents of that view cite evidence of aggression in modern primates, fossil evidence of hunting by early hominids, and anthropological studies of war and violence in human tribal and hunter-gatherer cultures.

Yet fossil evidence indicates that *Australopithecus afarensis*, a 1.2-meter-tall hominid that many think evolved into *Homo sapiens*, had no stone tools or weapons with which to defend itself, no fire to cook meat, and no sharp teeth to eat it. For millions of years, Sussman contends, *A. afarensis* was stalked by numerous and large predators, including a now-extinct dog as big as a bear, saber-toothed tigers, hyenas, and crocodiles.

At the meeting, Sussman noted that about 5% of *A. afarensis* fossils show evidence of having been eaten, such as holes in skulls that fit the teeth of ancestral leopards. Modern large predators take about the same percentage of prey species, including chimpanzees and gorillas. What's more, humans are still hunted by crocodiles in Africa, tigers in India, brown bears in Tibet, and cougars in California.

Cat chow? A leopard's teeth fit perfectly into this hominid's skull.

Because *A. afarensis* did not have physical defenses with which to ward off predators, it was forced into groups, Sussman argues. He notes that all modern primates that are active during the day and are preyed upon live in groups. Such groups have more eyes and ears with which to spot predators and more individuals to mob or confuse them. Living in such defensive groups ultimately led early hominids to cooperate and socialize more fully, says Sussman, who details his theory in the recent book *Man the Hunted: Primates, Predators, and Human Evolution*.

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Others have argued that group hunting or warfare led hominids to develop those traits. "You can't go from the observation a species is preyed upon to anything specific about their social relationships," says evolutionary ecologist Richard Wrangham of Harvard University. Primatologist Frans de Waal of Emory University in Atlanta, Georgia, is more receptive to Sussman's theory. While noting that violence and war have probably always occurred, he stressed that "we are also a species marked by high levels of cooperation [and] conflict resolution, … and it is time science started paying more attention."

–DAN FERBER

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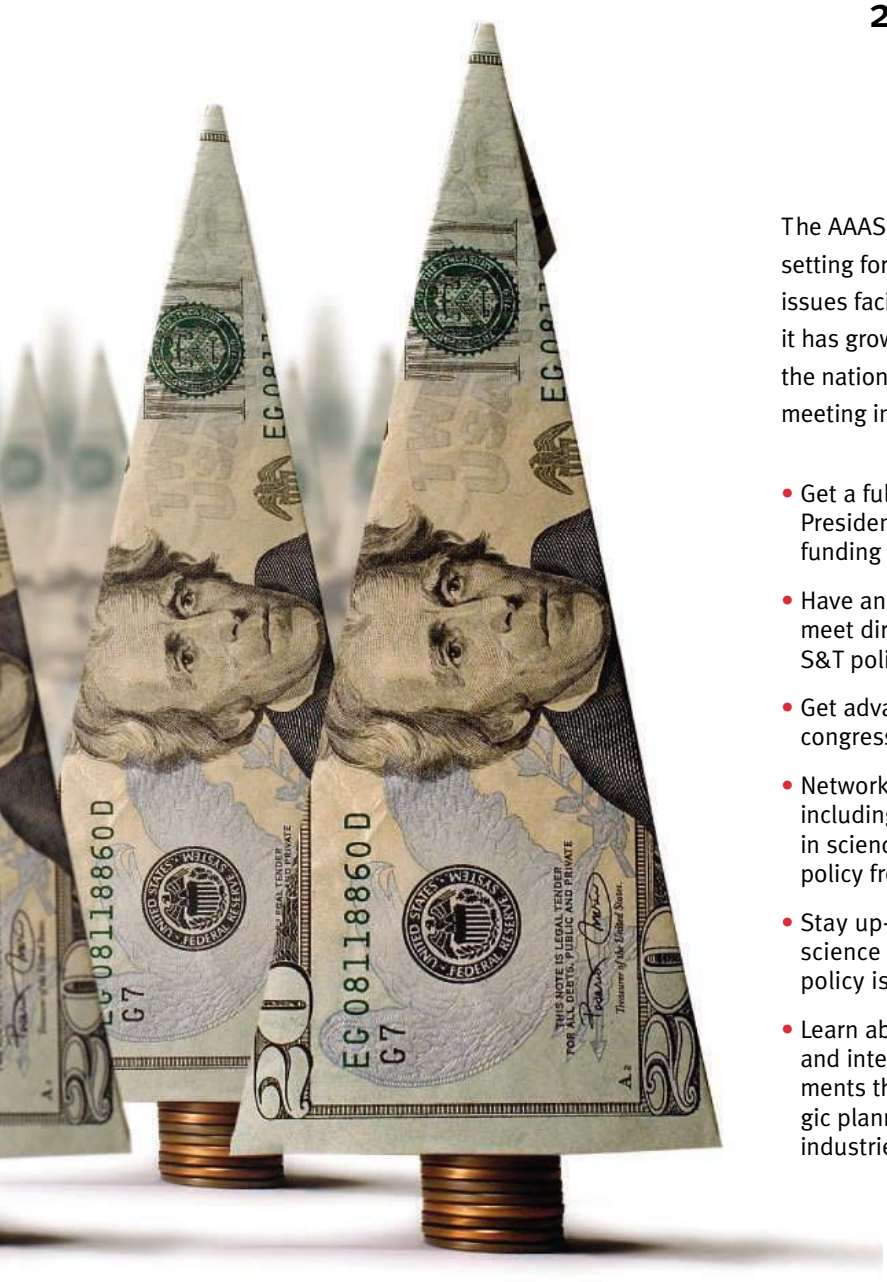

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EDITED BY YUDHIJIT BHATTACHARJEE

Pioneers

CLEANING HOUSE. Sam Ciurca was a teenager when he found his first fossil sea scorpion. "I saw two eyes staring at me," he recalls. "That one rock changed the course of everything." For the next 40 years, Ciurca spent evenings and weekends collecting these extinct arthropods, called eurypterids. A chemist for Kodak in Rochester, New York, Ciurca even found the world's largest eurypterid—1.3 meters long—now on display at the Paleontological Research Institution in Ithaca, New York.

The collection eventually filled his house and garage. But last fall, the several thousand specimens were moved to Yale University's Peabody Museum. The 65-year-old Ciurca donated most of the fossils, save for a few that earned him a "modest" sum to continue his hobby. Yale's Derek Briggs has brought in postdoc Erik Tetlie (pictured, right) to study the collection. "It's not in drawers hidden away forever," Ciurca enthuses.

APPOINTMENTS

FROM MD TO NYC. Allen Spiegel is vacating the top job at the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) to become dean of the Albert Einstein College of Medicine of Yeshiva University in New York City. An endocrinologist with 32 years at the $1.7 billion NIDDK, Spiegel has led the fifth largest National Institutes of Health (NIH) institute since late 1999. An NIH spokesperson on human embryonic stem cell research, which he calls "unequivocally essential," Spiegel briefed President George W. Bush before the 2001 speech that set limits on the number of federally funded cell lines. "It's a loss that he's leaving," says Lawrence Soler, vice president for government relations at the Juvenile Diabetes Research Foundation in New York City.

Spiegel takes over next month from Dominick Purpura, whose 22 years make him the longest-serving dean of a U.S. medical school. A priority will be moving clinical findings into practice in the school's north Bronx community. NIDDK Deputy Director Griffin P. Rodgers moves up on an acting basis.

HONORS

ENGINEERS FIRST. Two members of the second-term Bush Administration have been elected to the National Academy of Engineering (NAE). The selection of Energy Secretary Samuel Bodman and NASA Administrator Michael Griffin, both appointed since President George W. Bush was reelected in November 2004, has little to do with their current political posts and everything to do with their past scientific accomplishments, says NAE President William Wulf.

Bodman is a chemical engineer and former Massachusetts Institute of Technology professor whose "engineering talents" transformed Boston-based Cabot Corp. from a carbon-black producer to a broader materials company, says Wulf. Griffin, an aerospace engineer, is being honored for space experiments conducted more than 15 years ago. NAE has previously elected individuals with political backgrounds, Wulf notes, including Senator John Sununu (R–NH), a former White House chief of staff under George H. W. Bush, and former NASA administrator Daniel Goldin. For the full list of this year's 85 inductees, visit www.nae.edu.

DEATHS

THE COST OF WAR. Judges, clerics, and soldiers are not the only targets in strife-torn Iraq. At least 60 scientists have been killed in the past 3 years through assassinations, roadside bombings, and random attacks, according to Iraqi researchers living in Jordan.

That list, says Iraq-born neuroscientist Karim Alkhadi of the University of Houston, Texas, illustrates the mortal danger facing Iraq's academics and the university system they work for. The threat is obscured by the war itself: "It is difficult to ascertain the motives for each killing—if there is any," says Alkhadi, who left Iraq with his family in 1980. "But there seems to be an anti-intellectual campaign afoot."

Alkhadi, whose nephew was among several men recently gunned down in Baghdad, worries that the killings could obliterate the already fractured Iraqi educational system. Among the dead are some of the country's foremost researchers, including Baghdad University nuclear physicist Majeed Hussein Ali; Asaad Salem Shrieda, dean of engineering at Basra University; and Bassem al-Mudares, a chemistry professor at Tikrit University.

Movers >>

A SECURE START. Charles McQueary is stepping down next month after 3 years as the founding head of the science and technology directorate at the U.S. Department of Homeland Security (DHS). McQueary says he felt DHS Secretary Michael Chertoff, who took over last year, "should have the chance to hire his own people" but that he's very proud of what his $1 billion, 380-person office has accomplished, including funding five university-based centers of excellence and supporting more than 300 homeland security scholars and fellows.

"I thought we'd need to spend more time helping universities set up research programs in the field, but I learned that there is a lot of expertise already in place," he says. He also expects that the share of his office's budget devoted to basic research, now only 2%, will grow as more academics explore questions that relate to homeland security.

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LETTERS

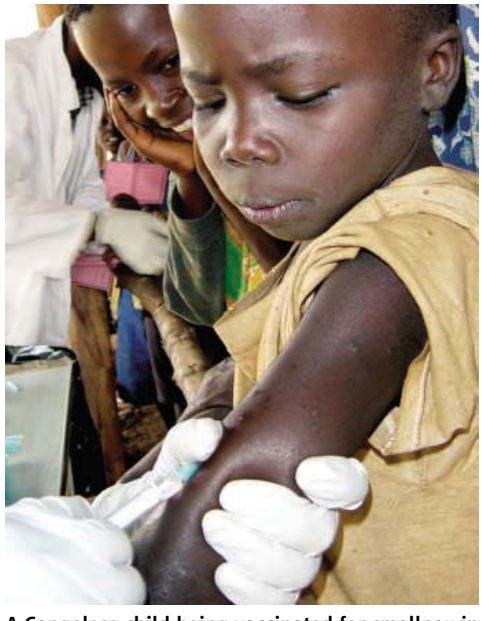
edited by Etta Kavanagh

Making Sure Public Health Policies Work

I TRUST IT WAS WITH TONGUE IN CHEEK THAT Jon Cohen, in his article on the new world of global health, referred to "an obscure 1978 health conference in the USSR" ("The new world of global health," News Focus, 13 Jan., p. 162). That "obscure conference" was the World Health Organization's Alma Ata conference on Primary Health Care.

Among its simple prescriptions was that, to ensure effective health care for people in poor communities, health structures must be built in those communities. "Smart weapons" have their limitations, in health as in the military, and the most effective medicine is invariably rooted within a society, not parachuted in from outside.

This lesson is habitually forgotten by the experts, which is why we now find that "shortages of trained health-care workers mean that those drugs that are available may not be used properly."



A Congolese child being vaccinated for smallpox in a refugee camp in Burundi.

Unfortunately, health policy in poor countries is too often directed by people who do not live in them, for whom these are academic matters. One need to look no further than rural China to understand what happens when effective community-based health care systems are allowed to collapse.

All the wonderful and worthy global health initiatives will work only if there are local systems through which they can be delivered. To achieve our goals, we need to keep a little space on the podium with all the great and the good (and some small change) for that most effective of all solutions, the well-supported primary health care worker.

MIKE MULLER

Visiting Research Fellow, School of Public and Development Management, University of the Witwatersrand, WITS, 2050, South Africa.

THE NEWS SUMMARY OF THE CURRENT INTERnational efforts to control infectious diseases was both timely and interesting ("The new world of global health," J. Cohen, News Focus, 13 Jan, p. 162). There is no doubt that the agenda for improving health in poor countries is moving forward. We are concerned, however, that the approaches outlined focus on technology and research, while ignoring evidence from public health care systems. The provision of adequate health care services relies heavily on material and human infrastructure, as well as active community involvement. Evidence of an approach that works can be found in the experience from Cuba over the last four decades (*1*). With a focus on training and education, universal provision of primary care, vaccination campaigns, and community mobilization, Cuba has achieved goals that remain elusive for the countries discussed in the article. Infant mortality is currently lower in Cuba (6 per 1000 live births) than in the United States (7 per 1000), and life expectancy is 77 years (*2*, *3*). Many infectious diseases, including polio, measles, rubella, mumps, and diphtheria, have been eliminated, and substantial progress has been made in reducing the cardiovascular disease burden (*4*). The Cuban international aid program has placed 24,000 physicians in African and Latin American countries that have seen their own health personnel leave for Europe and the United States (*5*, *6*).

Resources expended in global health could be better utilized if evidence from the Cuban health care system were incorporated into their appropriation. Unfortunately, the opportunity to learn from the Cuban model may be another of the victims of the half-century U.S. blockade of Cuba.

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Linking Bats to Emerging Diseases

A SARS-LIKE CORONAVIRUS FOUND IN 13 OF 46 horseshoe bats (genus *Rhinolophus*) ("Bats are natural reservoirs of SARS-like coronaviruses," W. Li *et al.*, 28 Oct. 2005, p. 676) led A. P. Dobson to conclude that these bats "have now been officially recorded as the natural reservoir host of the coronavirus (SARS-CoV) that causes severe acute respiratory syndrome…" ("What links bats to emerging infectious diseases?," 28 Oct. 2005, p. 628). He also said that SARS-CoV "almost brought the burgeoning

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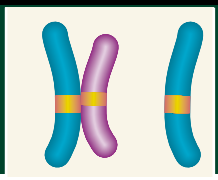
Counting X chromosomes
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Jurassic mammal education
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economy of Southeast Asia to its knees…," apparently on the basis of an unsubstantiated report (*1*). Li *et al.*'s data confirm that horseshoe bats are a reservoir for a coronavirus related to SARS-CoV found in humans and the virus isolated from palm civets, but this does not make them the reservoir for SARS-CoV.

Dobson's linking of bats to emerging diseases caused by Ebola and Marburg viruses is speculative and does not agree with published literature [e.g., (*2*)]. The assertion that direct transfer of Nipah virus from bats to humans occurred in Bangladesh and that human consumption of the pulp of fruit mouthed by bats is a route of these infections is not supported by the paper Dobson cited [i.e., (*3*)]. Ejected pellets of fruit bats consist of indigestible (by the bats) fiber and seeds with little nutritional value for animals (e.g., palm civets) unable to digest cellulose. Insectivorous bats cull indigestible insect parts, but we know of no evidence that this contaminates culled parts with bat saliva, let alone viruses.

People must be concerned about SARS and other emerging infectious diseases and the roles that bats and other animals may play in their epidemiology. When public health is the focus, however, those concerned for conservation of wildlife (including bats) must insist on careful consideration of documented facts rather than speculation and unsubstantiated statements.

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Response

FENTON AND COLLEAGUES RAISE SOME INTEResting points in their Letter. The fact that 13 of 46 horseshoe bats are seropositive to SARS certainly convinced me that horseshoe bats are likely to be an important reservoir of SARS. There are numerous economic analyses of the impact of SARS on the economy of Southeast Asia and Canada; I cited the most comprehensive of several possible references. There have now been outbreaks of Nipah virus in Bangladesh in each of the last 5 years—since detailed surveillance began. In all cases, fruit bats are implicated as the natural reservoir (*1*).

When we have visited the primary sites for the outbreaks of Nipah virus in Ipoh, Malaysia, the ground under the fruit trees shading the pig farms was littered with partially eaten fruit often bearing bat teeth marks. We also found similar partly eaten fruits under colonies of fruit bats south of Cairns, the site of a more recent Hendra virus outbreak. All of this makes me think it likely that bats do occasionally drop partially eaten food to the ground and that this food is occasionally eaten or ingested by other species: palm civets, pigs, horses, foxes, etc. I agree with Fenton *et al.* that rejected, or accidentally dropped, food items are unlikely to be major items in the diets of these novel host species, but interspecific transmission of most pathogens is a rare event. A recent paper partially confirms that bats are the likely reservoir hosts for Ebola virus (*2*). A similar mechanism can be postulated for the route of transmission of Ebola from bat to fruit to primate.

The main point of my Perspective is that I think it is crucial that we understand a lot more about the way that bats manage the interesting pathogens that infect them. This wish extends to a diversity of other hosts harboring pathogens that have a distant possibility of entering either human populations or our domestic livestock. Bats have many adaptations that have allowed them to adapt to a completely novel life-style when compared with other mammals (*3*). Adaptations such as torpor, a highly venous alimentary canal, flight, and a reduced skeleton provide important and different selection pressures on the pathogens that have to persist in populations of bats. The fruit-eating bats implicated as the reservoirs of SARS and Ebola have diets that are very low in nitrogen. Does this have any bearing on their inability to withstand infection by these pathogens? The major bat radiations occurred around 50 to 60 million years ago (*4*, *5*), significantly earlier than those of the other mammalian groups from which most pathogens of humans and domestic livestock evolved. This suggests there is a great need to search for novel regions in the immunological genes of the major bat families and to better understand the natural dynamics of infectious diseases in bats.

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Voucher Specimens for SARS-Linked Bats

WE READ WITH INTEREST THE REPORT "BATS are natural reservoirs of SARS-like coronaviruses" by W. Li *et al.* (28 Oct. 2005, p. 676). These authors and others (*1*) have identified bats in three genera as reservoirs of SARS-like coronaviruses (SL-CoVs), raising the possibility that SARS-CoVs arose among these or other bats. An omission in these papers is that no systematic attempt was made to preserve voucher specimens (*2*).

Properly vouchered specimens of reservoir hosts are a sine qua non in a research program whose goals are the accurate understanding of disease emergence and the ability to forecast disease risk (*3*, *4*). In the present instance, this basic standard was not followed and, thus, there is little that can be done to verify species identifications; this is especially troublesome when new data indicate that in SARS and other diseases, some individuals transmit more infection than predicted by homogeneous null models (*5*, *6*). Bat systematics is a dynamic area of research, and classifications change accordingly. This is especially the case in *Rhinolophus*, a group where species identification cannot be based solely on external morphological characters (*7*). Voucher specimens and associated data could be used for genetic validation of species identification and comparison to other forms

SARS has been found in horseshoe bats.

CREDIT: DK LIMITED/CORBIS

from Africa, Europe, and the Southwest Pacific. This would provide the potential for global prediction of SARS-like viruses.

We submit that work in epidemiology of infectious zoonotic disease is strengthened by following four steps: (i) depositing voucher specimens of all collected species in a local or international museum of natural history; (ii) preserving skin snips from all individuals, in alcohol or other preservative; (iii) identifying these samples by numbers so they can be cross-referenced to individual vouchers; and (iv) reporting these numbers in print.

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Response

WE THANK SALAZAR-BRAVO *ET AL.* FOR RAISING the important issue of accurate species identification in studying wildlife reservoirs for emerging infectious diseases. We agree that the collection and deposit of voucher specimens is extremely important in biological studies, including those of wildlife epidemiology. Our group has done the following to ensure that specimens were archived and that the bats captured were correctly identified: (i) We deposited specimens of each species (morphological type) captured during this study in the Institute of Zoology, Chinese Academy of Sciences, Beijing. (ii) In addition to morphological characterization, the identification of *Rhinolophus* species was supported by DNA sequence phylogeny. (iii) In a paper currently submitted to a peer-reviewed journal, we describe the DNA sequence phylogeny of this group of bats in China. The molecular data support our morphological identification. (iv) We have undertaken a long-term study of the diversity of bats in China as part of a Darwin Initiative–funded project. (v) We did not submit each individual bat that tested positive because of conservation and biosecurity issues; however, samples of blood from each bat are deposited at the Institute of Virology, Wuhan, China, as well as at the Australian Animal Health Laboratory, CSIRO, Australia. These institutions are able to safely store samples that potentially contain lethal infectious agents.

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Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 6 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

TECHNICAL COMMENT ABSTRACTS

Comment on "On the Regulation of Populations of Mammals, Birds, Fish, and Insects" I

Wayne M. Getz and James O. Lloyd-Smith

Sibly *et al*. (Reports, 22 July 2005, p. 607) concluded that density dependence acts far below the carrying capacity in most animal populations. We argue that the authors confused discrete and continuous models, that their best-fit models cannot explain observed oscillations, and that their estimation procedures appear biased. They also neglected trophic and migratory processes, which we demonstrate could underlie their empirical findings.

Full text at www.sciencemag.org/cgi/content/full/311/5764/1100a

Comment on "On the Regulation of Populations of Mammals, Birds, Fish, and Insects" II

Joshua V. Ross

Sibly *et al*. (Reports, 22 July 2005, p. 607) recently estimated the relationship between population size and growth rate for 1780 time series of various species. I explain why some aspects of their analysis are questionable and, therefore, why their results and estimation procedure should be used with care.

Full text at www.sciencemag.org/cgi/content/full/311/5764/1100b

Comment on "On the Regulation of Populations of Mammals, Birds, Fish, and Insects" III

C. Patrick Doncaster

Stochasticity in time series explains concave responses of per capita growth rate to population size. The gradients with the natural log of population size have more biological importance because they measure strength of density compensation. Its weakening with increasing body size across taxa (Sibly *et al*., Reports, 22 July 2005, p. 607) is consistent with slower responses in ascent than descent toward carrying capacity.

Full text at www.sciencemag.org/cgi/content/full/311/5764/1100c

Response to Comments on "On the Regulation of Populations of Mammals, Birds, Fish, and Insects"

Richard M. Sibly, Daniel Barker, Michael C. Denham, Jim Hone, Mark Pagel

The technical comments by Getz and Lloyd-Smith, Ross, and Doncaster focus on specific aspects of our analysis and estimation and do not demonstrate any results opposing our key conclusion—that, contrary to what was previously believed, the relation between a population's growth rate (*pgr*) and its density is generally concave.

Full text at www.sciencemag.org/cgi/content/full/311/5764/1100d

CORRECTIONS AND CLARIFICATIONS

News Focus: "A timely debate about the brain" by Y. Bhattacharjee (3 Feb., p. 596). The story did not mention Matthew Matell's affiliation; he is a researcher at Villanova University in Villanova, Pennsylvania. Also, Matthew Leon was Michael Shadlen's postdoc, not his graduate student.

News Focus: "China: healing the metaphorical heart" by G. Miller (27 Jan., p. 462). The Chinese characters depicted in the illustration on page 462 were identified in the caption as yiyuzheng, a technical term for depression, but they actually represent youyuzheng, a more commonly used term for depression.

Editors' Choice: "The grandmother effect" (20 Jan., p. 305). The last sentence of this item is incorrect and should have been deleted.

HISTORY OF SCIENCE

Ecology Beginning with the Botanists

Nancy Slack

The Evolution of American Ecology, 1890–2000

by Sharon E. Kingsland

Johns Hopkins University Press, Baltimore, MD, 2005. 325 pp. $50, £33.50. ISBN 0-8018-8171-4.

Tucson, Arizona, was an isolated little city surrounded by granite mountains in 1903, when the recently founded Carnegie Institution chose a flat-topped hill outside of town for the location of its Desert Laboratory. The laboratory is a focal site in *The Evolution of American Ecology, 1890–2000*, and Sharon Kingsland quotes William Hornaday's account of its origins: The Tucson Board of Trade took botanist Daniel MacDougal (the laboratory's future director) to the top of a mountain overlooking the site and told him, "All this shall be thine, and more, if thou wilt pitch thy tent herein, and become one of us" (*1*).

Pitch their tents the scientists did. In the early days, some lived in tents on the laboratory grounds, where a small community of families grew up. Wives often worked in the laboratory as well. It was a research community dedicated to basic research. Carnegie scientists could carry out well-funded long-range studies that state legislatures were not likely to support at agricultural stations. Former academics were free of teaching and administrative duties. They were lured to Tucson by the "promise of a fascinating landscape and intellectual freedom." Field and experimental studies, including long-term studies of plants in relation to the harsh desert environment, were encouraged. At this time of intellectual ferment—shortly after the rediscovery of Mendel's laws and the advent of Hugo de Vries's mutation theory—the scientists also conducted research in experimental evolution from MacDougal's neo-Lamarckian viewpoint.

Kingsland (a historian of science at Johns Hopkins University) describes this new type of research community and its work as a way of placing ecology in the context of the economic expansion of the country and the need for science to support that expansion. Thus even basic science was viewed as a "quest to expand human dominion over the land." In terms of range management, for example, good science would lead to rational methods whereas trial-and-error approaches had already led to disaster.

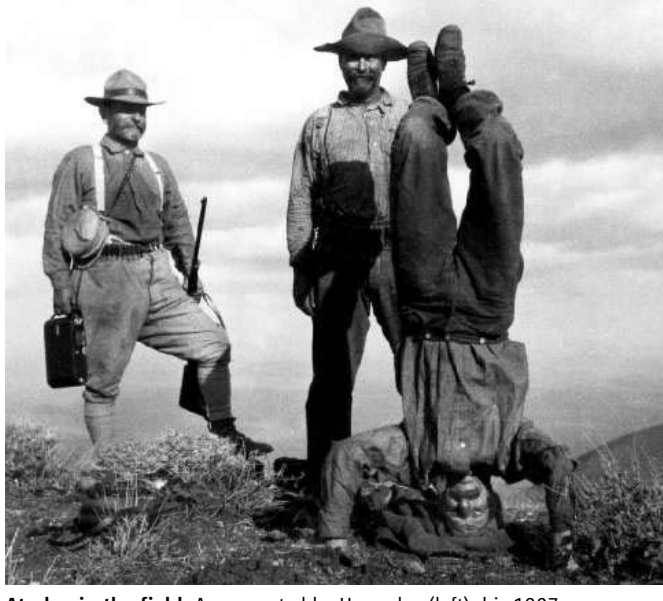
The author views the New York Botanical Garden as "midwife for the emergence of ecology in the United States," an idea that will be new to most ecologists. In the initial chapters of the book, she relates the history of the Garden. Later, she shows the many connections between it and the Desert Laboratory. For example, MacDougal had run the Garden's laboratories before moving to Tucson, and (despite being owned by the Carnegie Institution) the Desert Laboratory initially functioned as a satellite of the Botanical Garden.

Among the many researchers MacDougal recruited to the Carnegie Institution, the most influential was Frederic Clements. Already well known for a book on research methods in ecology that advocated the use of experimental, graphical, and statistical approaches (*2*), Clements wrote his magnum opus of ecological theory, *Plant Succession* (*3*), during his early years at the Desert Laboratory. He became the leading plant ecology theorist in pre–World War II America (*4*, *5*). His concept of the plant community as an "organism" and his deterministic view of plant succession held sway for almost 30 years, too long in the view of many ecologists. In the 1950s and thereafter, very different approaches—based on early work of Henry Gleason but developed by the Wisconsin group of plant ecologists and Robert Whittaker—took hold. These approaches viewed vegetation as a continuum in relation to changing climatic and other factors. New statistical and computer techniques such as ordination followed. Clements's views were repudiated, and at the 75th-anniversary meeting (1990) of the Ecological Society of America, a historical panel of eminent ecologists could find nothing good to say about Clements except that he did do experiments.

The Evolution of American Ecology is a rather misleading title, and professional ecologists may not recognize it as such. The first two-thirds of the book are largely about botany and plant ecology before World War II; there is little of the ensuing evolution of modern plant ecology. The last third turns to mainstream modern ecology and to concerns for the future of the field, a topic to which the author has given much thought.

The chapter "A Subversive Science?" makes particularly good reading. Beginning with an excellent short history of ecosystem ecology, Kingsland follows the conversion of ecology from a soft to a hard—or at least harder—science. Researchers—starting with G. Evelyn Hutchinson at Yale and his students Raymond Lindeman, Howard T. (Tom) Odum, and Robert MacArthur—developed more sophisticated, often mathematical theory. Tom Odum's doctoral thesis was on the cycling of strontium in the ecosystem—before the debate about the hazards of radioactive fallout. Physics, radioisotopes, and eventually computers and computer simulations came into play. Those who sought major funding from the U.S. Atomic Energy Commission (AEC) particularly required a connection to hard scientists, such as physicists studying radiation effects of nuclear testing.

Eugene Odum's 1953 *Fundamentals of Ecology* (*6*) was used, in its successive editions, by at least two generations of students. Several chapters by his brother Tom helped make the book more ecosystem-oriented and covered nutrient and other cycles, the geochemical approach pioneered by Hutchinson. Eugene Odum and his University of Georgia students used radioactive tracers in studies of field succession on lands around the Savannah River Atomic Energy Plant, South Carolina, and a salt marsh at Sapelo Island, Georgia. The Odum brothers also carried out a major project on a coral reef at Enewetak Atoll that had been a bomb-testing site. All of this research was funded by the AEC. Supported by the Office of Naval Research, Tom Odum also carried out a major ecosystem study in Florida. The substantial funding many ecologists received from these two organizations helped give rise to



At play in the field. As recounted by Hornaday (left), his 1907 expedition to Mexico's Pinacate region mixed science, comradeship, and adventure (*1*).

CREDIT: COURTESY ARIZONA HISTORICAL SOCIETY/TUCSON (AHS 70543)

The reviewer, the author of the forthcoming *G. Evelyn Hutchinson and the Invention of Modern Ecology*, is at the Biology Department, The Sage Colleges, Troy, NY 12180, USA. E-mail: slacknan@aol.com

ecology as "big science." Later, the National Science Foundation (NSF) supported large ecological programs, in particular the International Biological Program.

The relation of human ecology to ecology as a science has always been problematic. Kingsland discusses a few environmental classics, such as George Perkins Marsh's *Man and Nature* (*7*) and Rachel Carson's *Silent Spring* (*8*). Paul Sears dubbed ecology the "subversive science" because it provides grounds for "a continuing critique of man's operations within the ecosystem" (*9*). But many ecologists were long ambivalent about taking up human environmental issues.

That has changed over the last two decades. In the chapter "New Frontiers," Kingsland provides valuable insights on some current ecological concerns. An example is the Baltimore project, one of the two NSF-funded, long-term ecological research projects (LTERs) in an urban environment. For both long-term and interdisciplinary research, funding is difficult to obtain. Thus ecologists have been drawn to topics of interest to government funding agencies (e.g., radioactive tracers, productivity, global warming). In the past, little support has gone to human ecology per se. The Baltimore LTER, which the author has been following, is a human-centered research project involving social scientists as well as ecologists. It has practical goals for the city and people of Baltimore. Most readers, of whatever discipline, will find it a new approach to ecology. Kingsland offers an illuminating perspective on the ways it is accomplishing its goals and the ways in which it falls short.

I have touched on only some of the episodes that Kingsland covers in her account. *The Evolution of American Ecology* is not a comprehensive history of the field. Nonetheless, it is well worth consideration by ecologists, science historians, and anyone interested in how human ecology should be integrated with the biological sciences.

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10.1126/science.1123437

FILM: PLANETARY SCIENCE

Roaming Mars

Linda Rowan

If you've ever wondered what would happen if the National Aeronautics and Space Administration (NASA) teamed up with Walt Disney Pictures to make a movie, then go see the new IMAX film *Roving Mars*. The movie covers the adventures of the two Mars Exploration Rovers, Spirit and Opportunity, as they are bundled into pods by engineers at NASA labs, launched on Delta rockets, and parachuted to a bouncy landing. Spirit and Opportunity have spent more than two Earth years trundling across the surface of Mars, gathering data on rocks, soils, temperatures, and atmospheric conditions. Now those data come to life on the big screen with a little help from Disney and animator Dan Maas.

Roving Mars

by George Butler

Walt Disney Pictures, Burbank, CA, 2006. An IMAX film, 40 minutes. http://disney.go.com/disneypictures/rovingmars

The movie starts a bit slowly, with beautiful, if overused, images of faraway cosmic structures taken by the Hubble Space Telescope. The images look flatter on the IMAX screen than one might have hoped, and a nondescript narrator concludes the introduction by asking the age-old question "How did life begin?" After a global snapshot of Mars against a fake-looking background of stars, the film focuses on its real stars, the rovers and the scientists and engineers who have made their missions a great success. Thankfully, the narrator is frequently replaced with firsthand accounts and descriptions from the engineers and scientists. Geologist Steven Squyres, the leader of the rover science team, does much of the talking, casually dressed except for a fancy pair of cowboy boots. In one outdoor scene, he wears a black cowboy hat while standing over a rover to add the full affect to his space cowboy demeanor. Squyres does an excellent job enthusiastically and clearly conveying the rover technology and the geology. At one point he describes the acidic water that presumably once existed on Mars as "wine red pools under a pink martian sky."

Much of the film's first half, covering the final preparation of the rovers, takes place in clean labs—with engineers walking around in white puffy suits and white puffy hats. The close-ups of the rovers show some of the extraordinary complexity of the robotics and instrumentation. One scene shows a test of the six-wheeled rocker-bogie system in which a rover remains horizontal while its wheels hike over several rocks. Footage of the landing system design, particularly the parachute and air bags, includes a parachute test in a wind tunnel that looks fantastic in IMAX. Much of the footage in this part of the film was also used in the NOVA episode "Mars Dead or Alive," which goes into much greater depth about the design of the rovers and their landing system. I highly recommend watching that episode online or buying the DVD version if you want to learn more about the mission design.

The film's second half begins with the launch of the rover pod on a Delta II rocket. Extreme sound effects included in Philip Glass's score make you feel as if you are standing right next to the rocket. Animation produced by Disney shows each stage of the rocket jettisoning parts until the rover pod is spun up and popped off the uppermost stage to start its long coast to Mars. Glass can be forgiven for adding some extra sound in the near-vacuum of interplanetary space and for scaring some members of the audience (particularly younger ones) with the huge suddenness of every explosion and pop.

The animation and sound effects of the landing on Mars provide another highlight, but the best part of the film starts when the rovers begin bouncing on the planet's surface. Here the resolution provided by the rovers' cameras is combined with the realism of cutting-edge digital animation. The images of landscapes, rocks, soils, minerals, sunsets, and atmospheric haze are from collected data, whereas the rovers seen trundling over rocks, abrading rock surfaces, and scanning the martian sky are animations created by Maas Digital LLC. Seamlessly placing first the airbagged pod and later the rovers into the NASA images, Maas has created a surrealistically natural effect. Those who have been following the rovers will discover their favorite images are even bigger and bolder in IMAX.

The film says little about finding life on Mars, although it includes some discussion of the evidence for water that the rovers did discover. *Roving Mars* ends with a suggestion that the next generation of children will go to Mars, and Squyres concludes we will see their "bootprints in our rover tracks." Perhaps this is a little too ambitious even for a space cowboy like him. Nonetheless, the children in the audience were all smiling and wide awake when they left the theater.

10.1126/science.1125758

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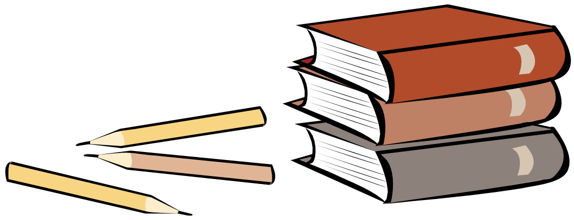
CREDIT: ROVING MARS/WALT DISNEY PICTURES

COLLABORATIVE PROGRAMS

Genome Consortium for Active Teaching (GCAT)



A. Malcolm Campbell,[1,2]*† Todd T. Eckdahl,[2,4] Edison Fowlks,[2,5] Laurie J. Heyer,[2,3] Laura L. Mays Hoopes,[2,6] Mary Lee Ledbetter,[2,7] Anne G. Rosenwald[2,8]

A supportive network of scientists and faculty brings sophisticated microarray experiments to the undergraduate lab and classroom.

Biological research has been transformed in recent years by substantial advances in efficient data accumulation. The transcription output for every gene in a genome now can be measured in an afternoon; before it might have taken years. However, the recent advances in technology have yet to be incorporated into many biology classrooms (*1*). Most undergraduates are taught the same way their instructors were taught, which seldom reflects leading-edge research practices. Training faculty in the latest research methods is not well supported on most campuses (*2*). Worse yet, when students with outdated undergraduate science experiences become primary and secondary school teachers, they condemn future generations to inadequate preparation for college. Today's teachers may also neglect the more quantitative aspects and increased interdisciplinary involvement of modern biology (*3–5*). Educational options that reflect quantitative, interdisciplinary, and technological trends would provide students with experiences that mirror today's scholarship.

GCAT in the lab. Undergraduates prepare samples and scan microarrays as part of their research at Davidson College.

CREDIT: DAVIDSON COLLEGE

We have developed the Genome Consortium for Active Teaching (GCAT) (*6*) to engage undergraduates in genomics experimental design and data analysis. GCAT faculty use DNA microarrays to bring the excitement of interdisciplinary research to students. Students discover the importance of quantitative data analysis, and the faculty are reinvigorated by the opportunity to learn new technology.

Origins of GCAT

GCAT was formed in 1999 with the intent of bringing genomics into undergraduate curricula, primarily through student research (*7*, *8*). Leading scientists donated materials and equipment. Undergraduates designed and performed experiments (see photograph above), mailed their microarrays for scanning, and then downloaded and analyzed their data (*9*).

Two limiting factors, long-term scanner access and a growing appetite for microarrays, were addressed by grant support and further donations from scientists (*10–12*). GCAT thus grew in size and expertise. GCAT supports free access to information and results through its Web site (*6*) and a listserv of more than 200 subscribers.

GCAT projects replaced student laboratory methods less prevalent in today's research, such as cloning and sequencing a gene and Northern blotting.

Rapid Growth

GCAT is committed to enabling any institution to adopt the use of microarrays in its undergraduate curriculum at affordable prices. To date, about 5000 undergraduates from 120 schools have used about 3400 microarrays. For the 2005–2006 academic year, GCAT provided more than 750 microarrays of nine plant, animal, and microbial species to students on 64 different campuses (*6*, *9*). Tested protocols and teaching aids are available from GCAT. Continued grant support (*11*) covers the cost of microarrays.

Schools pay a nominal fee to GCAT for microarrays and scanning. Students produce and hybridize their own probes. Other than the scanners, only standard molecular biology equipment is required; the software is free. The summer workshop costs, which are currently covered by grant support, are about $2300 per participant.

The number of interested faculty continues to grow. Although this enthusiasm is more a measure of the importance of the microarray method in molecular biology today than of GCAT itself, it also serves as a testament to GCAT's user-friendly format.

GCAT faculty use the microarrays in various ways. Some analyze existing data sets, such as the yeast diauxic shift data (*13*) that shows how yeast switch from one metabolic route to another. Other faculty members offer courses in which students collect their own microarray data. Students have studied the effects of environmental conditions on growth, aging in yeast, chromatin structure, and the cellular side effects of chemotherapy (*6*). Microarrays offer a view of the connections between different pathways in a cell in ways that are hidden by many other methods. For example, one student project looked for expression changes in DNA replication mutants and found cell wall assembly changes, thus linking cytokinesis to mitosis.

Dissemination Through Faculty Development

GCAT has sponsored data generation (wet lab) and data analysis (dry lab) workshops in various settings (*14*). Wet and dry lab sessions work best when they run 2 and 3 days, respectively. Participants learn data analysis using MAGIC Tool freeware (*15*). MAGIC Tool works on any computer platform and is designed to enhance student understanding of

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microarray and data analysis techniques.

In 2004, 35 faculty attended NSF-funded data analysis or combined data generation and analysis workshops at Georgetown University. Assessments demonstrated that combined training had a greater impact on undergraduate courses than the analysis workshop alone. The 23 who participated in the combined workshops reported that 800 undergraduates subsequently used microarrays (~35 students per teacher). In 2005, 64 faculty received microarrays. With similar rates, the microarrays might reach as many as 2200 undergraduates.

GCAT Students Participate in Various Aspects of the Scientific Process

85% hybridize probes to microarrays
78% produce cDNA probes
58% analyze their own data
53% design their experiments
25% analyze published microarray data
63% write a paper for course credit
35% present a poster of findings

Diversity

Historically black colleges and universities (HBCUs) are often left behind the technology curve. Two-thirds of attendees at the 2005 GCAT workshop at Morehouse College represented schools with substantial populations of underrepresented students, including African Americans, Native Americans, Hispanics, and nontraditional students attending community colleges. These populations are critical for diversifying the population of scientists in the United States. Faculty from biology, chemistry, mathematics, and computer science have attended GCAT workshops. GCAT activities have attracted diverse populations of students: 21% of GCAT students are non-Caucasian, 64% are female, 21% are majoring in a discipline other than biology, and 44% are interested in pursuing research careers in biology. GCAT implements BIO2010 recommendations (*1*) by teaching genomics through student research, which excites students across disciplines and ethnicities.

Keys to Success

GCAT's success is due to the people involved. The early GCAT faculty took a collective leap of faith by teaching genomics while simultaneously learning it themselves. Today's GCAT users can avoid much of the risk by taking a workshop before beginning with microarray analysis. GCAT faculty demonstrate their dedication by voluntarily leading the consortium's efforts (*16*). Working as a community maximizes efficiency and produces a sense of belonging to a larger effort that transcends a single campus.

Faculty and students participate in assessments of student comprehension, attitudes toward research, and demographic information. Anonymous, open-ended responses from students have been very enthusiastic. Selected comments from students include, "Microarray: GREAT! I am amazed that we can do this! Such an interesting concept yet simple enough to perform" and "What a powerful concept, microarrays. I greatly appreciated the opportunity to use what is quite possibly the most important tool in current analyses of gene expression."

Pre- and posttest results showed that GCAT courses produced significant improvement ($P < 0.001$) in students' abilities to design experiments and interpret data, areas often neglected in traditional teaching laboratories (see table). For example, students learned that whole-pathway changes are more reliable than individual gene changes. Students saw how spot identification must be quantitatively guided and how ratios are more informative than intensities. When faculty explained their learning goals, how they use GCAT resources, and the impact GCAT had on their ability to use microarray technology, they overwhelmingly indicated that they would not be able to do this work without GCAT resources and will continue to participate in GCAT activities.

When participants of the 2004 workshops were surveyed 1 year later, 80% (64% response rate) rated their experiences with the highest category on the survey. Sixty-one percent indicated networking with other faculty was very valuable. Faculty who had attended the combined data generation and analysis workshop altered an average of 1.6 courses to include the new content, whereas those who had attended only the data analysis workshop modified half as many courses (average 0.86). Faculty reported that their students showed an increased interest in mathematics as a result of microarray experiences. Faculty felt their teaching had improved and their classes were more interesting. One faculty member wrote, "… the presentation of this subject makes [students] realize and practice the close interaction biology/genetics has with other fields like mathematics. They enjoyed [being] introduced to a novel genetic technique. They said they can understand better, and can relate their class more to real life…when they watch [news about] health and advances in science." Another faculty member reported, "…many students have come back and said they got jobs or were assigned or allowed to do special projects in graduate schools because of their experience with microarrays."

Future Directions

GCAT wants to reach more faculty, especially at HBCUs, tribal colleges, Hispanic-serving institutions, community colleges, and small institutions. Regional workshops are being developed. GCAT is also working with high school teachers to develop a classroom and laboratory module on DNA microarrays (*9*).

Worrisome data suggest that students in the United States are falling behind students in other countries in the sciences. The National Assessment of Educational Progress "national report card" indicates only 18% of high school seniors were proficient or advanced in science in 2000 (*17*). Our educational system must prepare both future scientists and science-literate citizens for success in a world of continuing scientific and technological advances. The GCAT approach encourages faculty who focus on undergraduate teaching to become pioneers in incorporating the technological innovations of molecular biology. The GCAT community empowers faculty and students alike to solve educational problems (*1*–*5*) that seemed too big to tackle individually but were too important to ignore.

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Supporting Online Material

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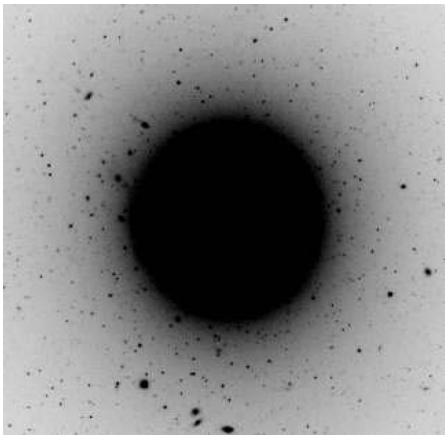
ASTRONOMY

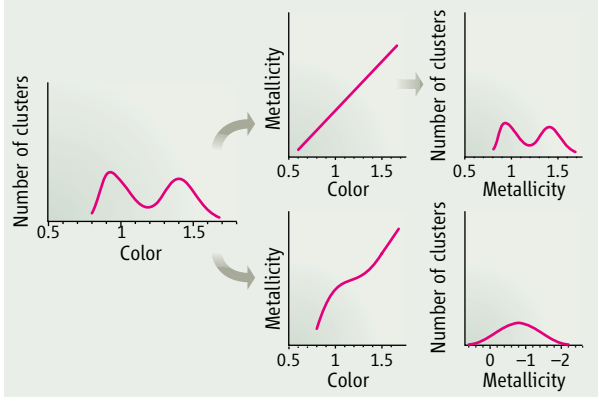
Sorting Out the Colors of Globular Clusters

The colors of the stars in globular clusters fall into two groups. Originally thought to result from stars with different ages, the colors can now be explained by the stars' composition.

Kenneth C. Freeman

Elliptical galaxies are believed to form from the mergers of a few smaller galaxies. Most ellipticals are surrounded by a cloud of very old globular star clusters (see the figure, upper left). As a feature of this merger origin, Ashman and Zepf (*1*) predicted that elliptical galaxies would have two distinct populations of clusters that appeared at different phases in the process of formation and chemical evolution of the parent galaxies. Over the last decade, the color distribution of the clusters in most ellipticals was indeed found to be bimodal. We know that the color of old stellar systems correlates with their metallicity (*2*), so the bimodal colors were interpreted as a bimodal metallicity distribution, and therefore as evidence for the predicted two populations of clusters. This bimodal color distribution was an exciting confirmation of the current ideas about galaxy formation, but this confirmation is now less secure. On page 1129 of this issue, Yoon *et al*. (*3*) show that a single cluster population with a broad monomodal metallicity distribution can have a bimodal distribution of color, because the relation between color and metallicity is not linear.





Color coordination. (**Top left**) The giant elliptical galaxy NGC 1399. The starlike objects in its outer regions are mostly globular clusters. (**Top right**) Example of a nearby galactic globular cluster NGC 6093. (**Bottom**) The observed color distribution of globular clusters (left) can be explained in two different ways. If the metallicity-color relation is linear, then the metallicity distribution in the cluster must be bimodal (top). Yoon *et al*. show that a nonlinear metallicity-color relation requires only a monomodal metallicity distribution (bottom).

Most galaxies contain some globular star clusters, which are dense, nearly spherical collections of typically 10^5 to 10^6 stars (see the figure, upper right, for an example). The Milky Way has about 150 globular clusters, whereas some of the largest elliptical galaxies have more than 20,000. The globular clusters in the Milky Way are all very old, with ages up to about 13 billion years. Their metallicities range from –2.5 for clusters in the halo of our Galaxy to near zero for the most metal-rich clusters of the galactic bulge. The low metallicities of the halo clusters show that they formed very early in the life of the Galaxy, before much of the chemical-element building had taken place.

We do not yet understand how these dense and massive clusters form. What was so special about the conditions early in the life of the Galaxy that led to their formation at that early time? Although globular clusters are not forming in the Milky Way at the present time, they are forming in violently interacting galaxy pairs like the Antennae system, in which two spirals are in the late stages of merging. The interaction has compressed the gas and stimulated much star formation, and the conditions in the interstellar gas are clearly right for the formation of young globular clusters.

For astronomers interested in galaxy formation, one of the most exciting discoveries of the last decade was the bimodal color distribution of the clusters in the bright elliptical galaxies (*4*). The metallicities of old clusters were already known to correlate with their colors (although the precise form of the color-metallicity relation was not well established observationally). From the time of its discovery, the bimodal color distribution of the clusters in elliptical galaxies was interpreted as a bimodal metallicity distribution. Typically, one mode has a metallicity around –1.5 and the other mode, a metallicity around 0. Both modes of clusters are old (>10 billion years).

Astronomers found this exciting because the presence of two cluster subsystems of different metallicities was consistent with the picture of elliptical galaxies forming from mergers. The metal-poor subsystem would be associated with the formation of the individual galaxies that later merged to form the elliptical. The metal-rich mode would be produced in the phase of rapid star formation during the merger process, from gas that had been already chemically enriched in the individual galaxies (as in the Antennae and other present-day merging spirals) (*5*).

But there is a catch. The interpretation of the bimodal colors depends on the true shape of the color-metallicity relation. Yoon *et al*. have made new theoretical stellar population synthesis models for clusters, and have also collated observations from the literature (*3*). Their theoretical color-metallicity relations agree very well with the observations. They find an inflected S-shaped color-metallicity relation (see the figure, bottom). The nonlinearity of this relation is significant: It may undermine the idea of two populations of globular clusters in ellipticals. A smooth distribution of metallicity (for example, a broad

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monomodal Gaussian distribution peaked at a metallicity of −0.8) is consistent with a bimodal distribution in color.

The reason for the nonlinearity of the color-metallicity relation goes back to the evolution of stars of different metallicities. Old evolved stars pass through a helium-burning phase (the horizontal branch of the Hertzsprung-Russell diagram), which is predominantly blue at low metallicities and becomes rapidly redder as the metallicity increases from −1.0 to −0.5. The mean colors of the less-evolved giant and dwarf stars also become redder at higher metallicities, again in a nonlinear way.

Yoon *et al.* also sort out another aspect of cluster color. The fraction of clusters in each color mode, and the mean colors of the modes, are observed to vary with the brightness of the host galaxy. These variations are easily understood in the Yoon *et al.* picture. Brighter ellipticals have higher mean metallicities than fainter ellipticals; this has been known for decades. Yoon *et al*. show how the projection of different metallicity distributions affects the predicted color distribution. As the mean metallicity decreases, the fraction of clusters in the blue mode increases, and the colors of both modes become bluer, just as observed. Similar variations within individual ellipticals can also be understood simply as a consequence of the internal radial gradients of metallicity that have also been known for many years.

The conclusion from the argument of Yoon *et al*. is that two separate epochs of globular cluster formation in ellipticals may not be needed. A single broad distribution of cluster metallicity can produce a bimodal color distribution. This makes sense because broad distributions of metallicity arise naturally in galaxies, from their continuous chemical evolution. Although the results of Yoon *et al*. do not exclude the merger origin of ellipticals, color bimodality may no longer be strong evidence for the two epochs of cluster formation that were predicted in the merger picture.

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PHYSICS

Implementing a Quantum Computation by Free Falling

Quantum computers may become more powerful than conventional computers, especially at solving hard problems. Finding efficient ways to tackle such problems turns out to resemble understanding the path taken by a falling object.

Jonathan Oppenheim

There are a number of arenas where quantum resources outperform their classical counterparts, but this improvement is particularly impressive in the theory of computation. Quantum computers can efficiently solve problems that are believed to be unfeasible on a classical computer, as they would need to run exponentially longer. What type of programs can be run on a quantum computer is a question that Nielsen *et al*. attack on page 1133 of this issue (*1*). Currently, we have only a handful of quantum algorithms, of which the most noteworthy are Shor's factoring algorithm (*2*) and Grover's search algorithm (*3*). To further our understanding, one of course wants to find more problems that can be solved faster on a quantum computer, and although progress has been made, this has proven to be a difficult task.

Although it is doubtful, it could even be that quantum computers can solve all problems in the class NP—those problems whose solutions can be efficiently checked on a classical computer (*4*). If such a thing were true, it would have radical implications not only for physics but for human thought in general. We believe that writing a great poem is more difficult than recognizing one, because many can do the latter but few the former. Likewise we believe that discovering a new theory of nature, which seems to require genius, is much harder than checking the correctness of the theory, a task that many are capable of. Yet at the moment we don't have a proof of the existence of problems whose solutions can be checked efficiently on a classical computer but not solved efficiently. Nor do we have a proof that quantum computers cannot solve such NP problems. Finding such an example is one of the great tasks of classical and quantum computer science.

What a computer does when it solves a problem is to implement a mapping between inputs to the computer and a set of outputs. Thinking of this in terms of a physical operation, one sees that the quantum computer is implementing a physical mapping from initial quantum states to final states. This physical mapping between states is what we call "unitary evolution" or sometimes

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Arriving at a solution. A quantum computation could be viewed as a path along a landscape of hills and valleys. The desired unitary evolution of states in the computation is represented by U. For the quickest path to the target unitary there exists a computation that runs at approximately the travel time. One wants to learn whether there is an efficient computation (polynomial time) or whether the computation is inefficient (exponential time).

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just a "unitary." We know that almost all unitaries cannot be efficiently implemented (*5*), but we don't have an example of one. Although understanding which unitaries can be efficiently implemented has proven difficult, one might be able to relate this problem to other problems that have been more thoroughly studied, and thus gain some greater insights. This is exactly what Nielsen *et al*. have done. They link a problem in Riemannian geometry—namely, finding the shortest path between two points—to the problem of deciding whether a unitary can be implemented efficiently. This allows ideas from each of these fields of research to inspire the other.

Given a family of unitaries U that act on registers of size *n* quantum bits (or qubits), we are interested in how long it takes a quantum computer to implement these unitaries. At each step of the computation, the computer performs one of some set of elementary interactions (called a gate). If the number of steps the computer uses scales polynomially in *n*, then we say that the computation is efficient. If the number of steps scales exponentially in *n*, then the computation is not efficient. Deciding whether the computation is efficient is a matter of decomposing the unitary into the smallest number of elementary gates. This is a daunting task, because there are all kinds of ways one can make this decomposition—how do we know that we have found an optimal one?

Nielsen *et al*., building on previous work (*6*, *7*), relate this question to geometry as follows. Imagine you are sitting at the center of a surface, and your goal is to reach some other point on it that represents your target U (see the figure). The authors show that if you take the shortest path to your target, then the time of your journey is close to the time it would take for a quantum computer to implement the unitary. If your journey takes a time that grows polynomially with *n*, then there exists an efficient implementation of the unitary (and vice versa). It works roughly like this: First put coordinates on the surface to guide you on your journey; the quantum computer will take the basis of your coordinate system to correspond to particular interactions it will apply during the computation. Next, the authors endow the surface with a metric, which tells us how to measure the time our journey to the target will take. The metric they choose causes clocks to run normally if we travel along directions that correspond to elementary interactions, but causes them to run very fast if we travel along directions that correspond to more complicated interactions involving more than two qubits. This forces us to avoid paths that travel in these directions if we wish to minimize our travel time. Now we want to take the shortest distance to our target—a geodesic. Geodesics are paths that a freely falling object would take, so to make our journey optimal, we should free-fall. We thus begin our journey by picking a direction and speed—but we must pick carefully if we hope to reach our target. In general, most geodesics will not pass through our target, and it may also be that there are many geodesics that pass through the target, forcing us to find the shortest one. Once we have found the shortest geodesic, Nielsen *et al*. then show that it corresponds to an implementation that approximates the desired unitary and that is of a length polynomial in the time traveled along the geodesic. This, coupled with a lower bound proof (*7*) (with the caveat that it pertains to exact implementation of the unitary without additional work space), completes the correspondence.

Finding the shortest geodesic between two points is of course a difficult problem; however, Riemannian geometry is a much more mature field than quantum computing and has the luxury of dealing with continuous paths, bringing with it all the power of differential geometry. One thus hopes that insights from it may yield some results in computation. Likewise, insights from computation might yield some surprises in Riemannian geometry. Proving that a particular unitary is difficult to implement is of great interest, so one would like to remove the caveats contained in the proof of the lower bound. Many questions are raised here. Because quantum states are closely related to quantum operations, both from a mathematical and an operational perspective, one wonders whether analogous relationships could be found for quantum states. One might also be able to relate the workings of classical computers to questions of geometry. The relationship between geometry and the implementation of unitaries promises to be, at the very least, stimulating.

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MOLECULAR BIOLOGY

"X"-Rated Chromosomal Rendezvous

Laura Carrel

Female mammals inactivate one of their two X chromosomes to ensure a dosage of genes equal to that of males who contain a single X. A brief union between the pair of X chromosomes may initiate this inactivation process.

The many ways in which men and women differ are attributed to the qualitative difference in the composition of their pair of sex chromosomes—XY chromosomes versus XX chromosomes, respectively. But difference in the number of X chromosomes also poses a potential problem. In mammals, most genes on one X chromosome are inactivated in females to equalize the "dose" of X-chromosome genes between XX females and XY males. Our understanding of this process remains incomplete, but two new reports, by Xu *et al.* on page 1149 of this issue (*1*) and by Bacher *et al.* (*2*), reveal an important facet of X chromosome behavior at the onset of X chromosome inactivation.

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The initial stages of this regulatory process are quite complex [reviewed in (*3*, *4*)]. Early in mammalian development, before X chromosome inactivation occurs, each cell must calculate the number of Xs and initiate inactivation only when more than one X is present. Furthermore, embryonic X chromosome inactivation is random—some cells initially decide to inactivate their maternally inherited X chromosome while others target the paternal X chromosome. Sequences regulating these counting and choice steps reside at the X inactivation center (*Xic*), a region on the X chromosome that includes three genes that encode noncoding RNA transcripts (*3*, *5*). The *Xist* gene, expressed only from the X chromosome that will be inactivated, encodes a structural RNA that coats the inactived X

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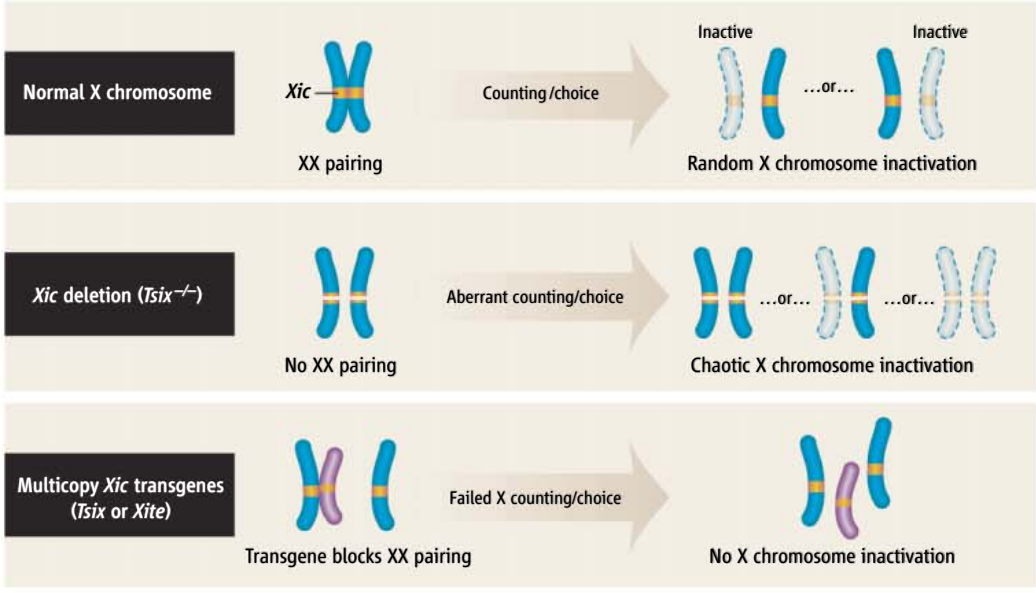
chromosome, and is essential for the inactivation process (*6*, *7*). Genes adjacent to *Xist*—its antisense partner *Tsix* and an upstream regulatory gene called *Xite*—ensure proper *Xist* expression. These genes, and adjacent DNA sequences, are responsible for the counting of X chromosomes and the subsequent choosing of which X chromosome becomes inactivated (*5*, *8*). Models suggest that these two steps involve chromosomal binding sites for factors that block the active X chromosome from initiating the inactivation process (*9*, *10*). Different binding sites are proposed to render the other X chromosome competent to undergo inactivation (*5*). Deletions within the *Xic* region and the insertion of *Xic* regions into non-X chromosomes as transgenes have narrowed the possible locations for these putative binding sites (*5*, *8*). A puzzling aspect of these models is how factor binding occurs in a mutually exclusive fashion, implying that there is communication between the two X chromosomes. One possibility is that such trans-sensing could occur through homologous chromosome interactions (*11*). This would predict that within each cell nucleus, critical regulatory sequences on the X chromosomes should lie in close proximity to each other to enable such chromosomal cross-talk.

To test this hypothesis, two independent groups (*1*, *2*) examined the spatial relationship between the two X homologous chromosomes, specifically the critical *Xic* region, during the onset and establishment of X chromosome inactivation. The *Xic*s on the X chromosomes were visualized by fluorescence in situ hybridization in mouse female embryonic stem cells. This model system recapitulates X chromosome inactivation in cultured cells, as one X chromosome undergoes inactivation upon embryonic stem cell differentiation. Strikingly, the *Xic* probes were often closely juxtaposed during early stages of embryonic stem cell differentiation when inactivation was being triggered (see the figure). No *Xic* "association" was observed at earlier or later time points of stem cell differentiation. To pinpoint the exact timing of this transient association, both groups measured *Xic* colocalization relative to well-characterized features of inactivation. *Xic* association is a very early event that takes place just before or at the same time as *Xist* RNA coats the X chromosome (*1*, *2*) and precedes the earliest chromatin changes that accompany inactivation (*1*). Therefore, this brief encounter between X chromosomes occurs at a critical early time point that suggests a functional role in the inactivation process.

These experiments showed that the *Xic* regions of each X chromosome colocalize, but how close is close? Are the *Xic*s really physically interacting, or could these observations simply reflect sequestration of both X chromosomes to a common compartment in the nucleus (*2*)? The latter seems unlikely, at least in total, as the association phenomenon is specific to the *Xic* region. Fluorescent probes that recognize DNA sequences outside of the *Xic* region were not colocalized at any stage of stem cell differentiation (*1*). Further, Xu *et al.* (*1*) used a technique called chromosome conformation capture to confirm the proximity of the two *Xic* regions. This technique physically cross-links DNA through associated proteins, and when the DNA is then cut and ligated, closely juxtaposed molecules are glued together. Such hybrid molecules spanning the two homologous X chromosomes were specifically detected at the same time that pairing had been visualized by fluorescence in situ hybridization. The homologous X chromosomes are indeed paired at least through their DNA-bound proteins.

Right time, right place—but does it mean anything? To find out, both groups analyzed pairing of X chromosomes in mouse embryonic stem cells carrying deletions in their *Xic* region (see the figure). A number of such deletion mutations that disrupted random X chromosome inactivation, also disrupted pairing between the two X chromosomes. Especially interesting is that this effect was seen for quite tiny deletions at the *Tsix* or *Xite* genes (*1*) (less than 4 and 6 kb, respectively). Furthermore, transgenes composed of *Xic* pieces inserted into non-X chromosomes could associate with X chromosomes and disrupt normal X chromosome pairing. Intriguingly, the same small *Tsix* or *Xite* regions that disrupt X-X pairing when deleted can partner with an X chromosome when they are relocated onto a non-X chromosome. Therefore, X cross-talk appears to be an integral step in the choice decision, and at least two nonoverlapping regions within the *Xic* are involved.

Additional *Xic* mutations and transgenes revealed that proper X chromosome behavior must conform to a specific set of "dating" rules, some of which have yet to be specified. Deletions indicate that pairing alone is not sufficient to ensure random X chromosome inactivation and that inactivation can occur without pairing, although it is not correctly regulated (*1*, *2*). Further, given that small *Tsix* or *Xite* transgenes can ectopically pair with an X chromosome (*1*), it is surprising that a larger transgene cannot do so, despite encompassing and extending beyond the entire *Xic* (*2*). Copy number likely explains such differences, as only a single copy of the large *Xic* region (460 kb) was inserted on the transgene versus multiple copies of the *Xite* and *Tsix* sequences. Do sequences out the *Xic* region mediate pairing? Normal pairing



Pairing, counting, choosing, and inactivating X chromosomes. (**Top**) Normal pairing of maternally and paternally inherited X chromosomes in a female mammalian diploid cell is mediated by the *Xic* region (yellow) in the X chromosomes. Random inactivation of one of the X chromosomes follows normal X-X pairing and counting/ choice steps. (**Middle**) The *Tsix*$^{-/-}$ deletion mutation alters pairing and affects counting and choice. "Chaotic" X inactivation results in cells that inactivate 0, 1, or 2 X chromosomes (*1*). (**Bottom**) A non-X chromosome bearing multiple transgene copies of *Xite* or *Tsix* sequences pairs with one X chromosome. This can disrupt normal X-X interactions.

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affinity may require multiple interactions that can also be attained by tandem insertions of a single sequence.

Many questions remain about the specific nature of this X chromosome rendezvous. In addition to identifying all the sequences required for pairing, we still need to know what the X chromosomes then do, how they do it, and what happens afterward, to ensure inactivation of just one of the pair. At X chromosome pairing, what process enables them to distinguish one from the other to achieve mutually exclusive choice? With so many questions left to answer, the future for this new relationship appears quite promising.

Chromosome cross-talk clearly adds a new dimension to the complex regulatory events at the initial stages of random X chromosome inactivation. Pairing is not unique to this inactivation process, as it coordinates monoallelic expression between other sites in the genome (*12*, *13*). Chromosome courtship may be a relatively common way to orchestrate gene regulation, and it will be important to see whether this coupling occurs in the same fashion as on the X chromosomes.

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PALEONTOLOGY

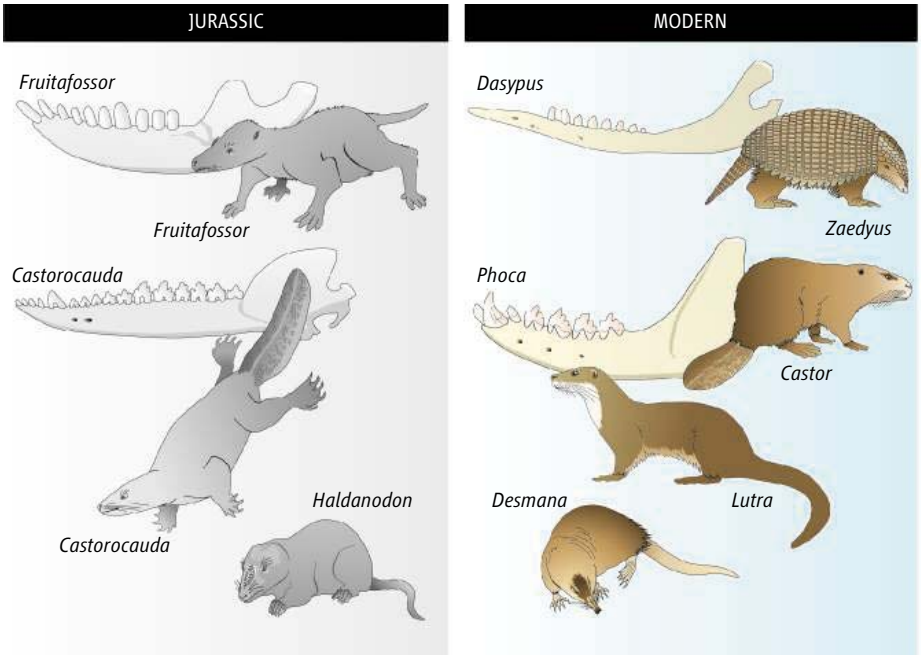
Early Mammalian Evolutionary Experiments

Before the extinction of the dinosaurs, most early mammals were thought to be small, nocturnal, and terrestrial. A new large Middle Jurassic fossil with fur and a beaverlike tail suggests that some were aquatic.

Thomas Martin

Mammals of the Mesozoic era (248 to 65 million years ago) generally are considered to be primitive, shrewlike creatures living in the shadow of the dinosaurs (*1*). Only after the extinction of the dinosaurs at the end of the Cretaceous era (144 to 65 million years ago) did they have a chance to explore a greater variety of ecological niches. During the adaptive radiation that began about 65 million years ago, mammals were able to invade all kinds of terrestrial environments, even the aquatic and aerial realms. Pushing back the mammalian conquest of the waters by more than 100 million years, Ji *et al.* (*2*) report on page 1123 of this issue a Middle Jurassic, 164-million-year-old skeleton with a beaverlike tail and seal-like teeth perfectly adapted for an aquatic lifestyle. This exciting fossil is a further jigsaw-puzzle piece in a series of recent discoveries, demonstrating that the diversity and early evolutionary history of mammals were much more complex than perceived less than a decade ago. It also impressively contradicts the widely held view that early stem representatives of modern crown groups (groups of organisms with living representatives) are generally primitive and unspecialized.

Until the mid-1970s, Mesozoic mammals were merely known by teeth and jaws that are informative for a systematic assignment but provide only limited insight into their paleobiology. The available fossil record drew a picture of most Mesozoic mammals as primitive and generalized insectivorous animals with no particular adaptations (*3*–*6*). The initial discoveries of more complete specimens with skeletons resembling that of the living opossum *Monodelphis* and cheek teeth with pointed cusps that are typical for insectivory supported this scenario (*7*, *8*). The only clue that the known fossil record might be just the tip of the iceberg of Mesozoic mammalian ecomorphological diversity (see the figure) was an incomplete skeleton of the Late



Unexpected diversity. The Late Jurassic docodont *Haldanodon* shows strong skeletal adaptations to a semifossorial life-style (*9*–*11*) similar to those of modern *Desmana* (water mole). The basal mammal *Fruitafossor* exhibits dental and skeletal convergences to modern digging xenarthran placentals (*12*) such as armadillos (*Dasypus, Zaedyus*). The new Middle Jurassic docodont *Castorocauda* (*2*) from Inner Mongolia possesses striking features for an aquatic life-style and combines skeletal, dental, and softpart characters of modern aquatic placentals such as beavers (*Castor*), river otters (*Lutra*), and seals (*Phoca*) [fossils and reconstructions redrawn from (*2*, *10*, *12*); artists: Figure Studio].

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Jurassic stem mammal *Haldanodon* from Portugal with striking similarities to that of modern digging and semiaquatic desmans (water moles) (*9*–*11*). However, fossoriality apparently was not uncommon among Jurassic mammals, as is indicated by the recently discovered enigmatic *Fruitafossor* from the Morrison Formation in Colorado (*12*). This stem mammal of uncertain ordinal assignment not only possesses a postcranial skeleton very similar to that of Australia's semifossorial echidna, but also perfectly imitates two extreme specializations that previously were thought to be unique to the South American xenarthrans (anteaters, armadillos, and sloths). These are peglike and rootless dentine teeth lacking enamel and a lumbar vertebral column that is stabilized by additional articulation facets (xenarthry), both adaptations for digging up and feeding on colonial insects (for example, termites).

The discovery made by Ji *et al*. enriches this growing Mesozoic mammalian zoo by adding a semiaquatic swimmer and fisheater. Most striking among the features of *Castorocauda* is the dorsoventrally flattened tail covered by small horn scales, remarkably like the modern beaver tail. Moreover, the exquisitely preserved fossil even presents other parts of the soft body such as hair and webbing of the hindfeet. Additional support for the aquatic adaptation comes from the anterior cheek tooth dentition, which closely resembles that of fish-eating seals. Interestingly, an aquatic life-style and fish diet were previously postulated for Early Cretaceous triconodonts from Morocco based on the shape of isolated teeth (*13*).

These exciting discoveries may just be a glimpse of what is to come. They dramatically demonstrate how many gaps remain in our knowledge of Mesozoic mammalian diversity. New fossils are essential to fill these gaps in the understanding of the evolutionary history of life that obviously was much more complex than perceived a decade ago. The potential of fossil-rich deposits like the Jehol group in Liaoning Province in China or the Jiulongshan Formation in Inner Mongolia is only just beginning to be exploited. So far only fragmentary, but all the more tantalizing, mammalian fossils have come to light from the Jurassic and Early Cretaceous of Gondwana (southern continents) (*14*–*16*), making these days an exciting time for mammalian evolutionary biologists. We stand at the threshold of a dramatic change in the picture of mammalian evolutionary history, and many chapters (*17*) of it will soon need rewriting.

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CELL BIOLOGY

The Stress of Finding NEMO

A cell's response to damaged DNA is triggered when two proteins leave the nucleus and converge upon a signaling complex in the cytoplasm. This activates a key transcription factor that then moves into the nucleus to promote cell survival.

Jiri Bartek and Jiri Lukas

To survive, all living organisms must cope with the adverse effects of "genotoxic stress," insults that constantly threaten the integrity and function of our genes. Such attacks come from environmental agents such as radiation, cigarette smoke, or chemical pollutants, and from the enemy within: the cell's own metabolic products that cause diverse lesions in the DNA. To deal with both the external and internal DNA-damaging agents, organisms have evolved mechanisms that slow down or block cell proliferation (so-called cell-cycle checkpoints), promote DNA repair, or eliminate damaged, hazardous cells by engaging a cellular suicide program. How cells make the choice between life and death in response to DNA damage is critical not only for the fate of each cell, but also for avoiding life-threatening diseases such as cancer (*1*). Despite efforts to better understand this fundamental cellular decision-making process, its molecular basis has until recently been obscure. Exciting work reported by Wu and colleagues on page 1141 of this issue (*2*) now provides mechanistic insights into the life-or-death choice in human cells exposed to the most deadly type of genetic damage, the DNA double-strand breaks.

It has been known for years that in response to double-strand breaks in DNA, cells activate the protein kinase ATM (ataxia telangiectasia mutated), a master regulator that phosphorylates (adds phosphate groups to) numerous other proteins and thereby modulates their functions in cell-cycle control, DNA repair, or cell death (*1*, *3*). Wu *et al*. have now identified a new substrate for ATM—NEMO (NF-κB essential modulator), a key modulator of the prosurvival transcription factor NF-κB (nuclear factor–kappa B) (*4*). In doing so, they have elucidated dynamic interplay between ATM and NEMO within the cell fate–decision machinery in response to DNA damage.

NF-κB and its activator IKK (IκB kinase) operate as an evolutionarily conserved signaling module that orchestrates inducible gene expression in diverse cell types and biological processes, allowing cells and organisms to adapt to environmental changes. Both the IKK activator complex and NF-κB are usually in a dormant, inactive state in the cytoplasm, poised to respond to signals from outside the cell (*4*). But the unorthodox ATM-NEMO–dependent mechanism (*2*) works "in the opposite direction," as a nuclear-to-cytoplasmic signaling cascade to activate the IKK–NF-κB system. Notably, although NEMO also participates in other modes of IKK activation (*4*), the ATM-NEMO interplay is signal specific, responding to DNA damage (*2*, *5*) but not to other types of stress or environmental stimuli that activate the IKK–NF-κB module through other mechanisms (cytoplasm-to-nuclear signaling cascades) (*4*).

Another fascinating feature of this mechanism is its very dynamic spatiotemporal regulation. Under normal, nonstressful conditions, both NEMO and NF-κB rapidly move back and forth between the cytoplasm and the nucleus. The apparent cytoplasmic localiza-

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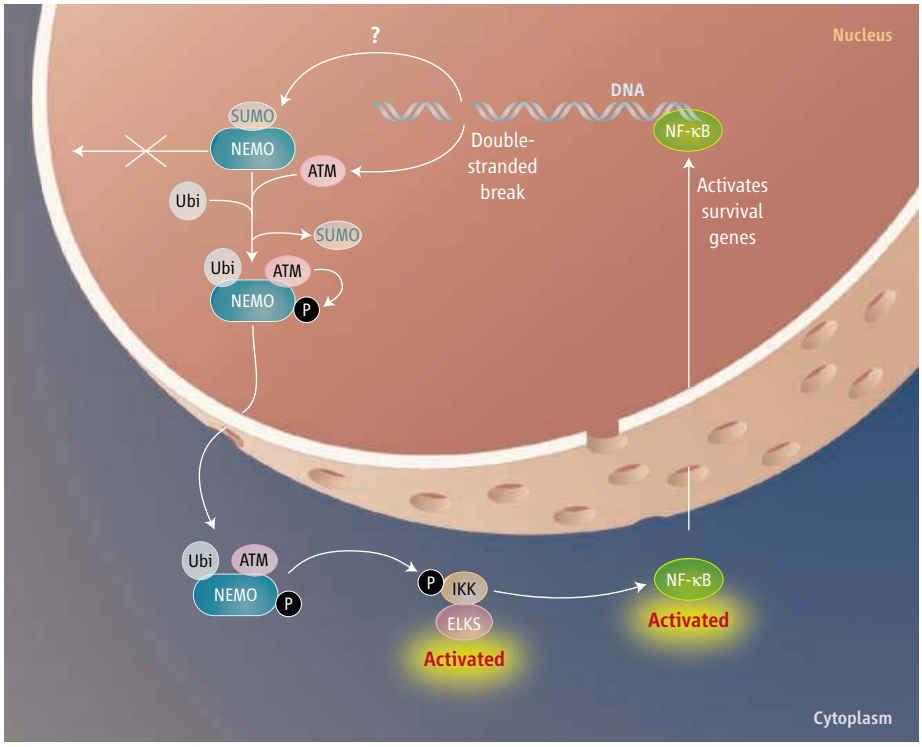
tion of NF-κB, for example, simply reflects the equilibrium state in a highly dynamic process. In response to DNA damage, the equilibrium states transiently change, first for NEMO and eventually for the active NF-κB. In addition, NEMO enters into multiple protein-protein partnerships whose prompt assembly or disassembly is crucial for activation of this signaling cascade and its timely biological impact (*2*, *5*–*7*). Thus, the emerging mission of NF-κB activation in response to DNA damage is the temporary activation of genes whose products help the cell to survive, providing a window of opportunity for DNA-repair pathways to correct the damage, and for the cell to return to its normal physiological state. This itself is remarkable, because among the many known targets of ATM (*1*, *3*), NEMO is one of the first such cell death–antagonizing substrates (*2*, *8*, *9*). Again, the transient impact of the ATM-NEMO–IKK–NF-κB pathway has a biological significance: In cells with irreparable DNA damage, the more durable, cell death–promoting signals (also fueled by ATM, which targets other substrates for a longer time than NEMO) may eventually prevail, and such genetically unstable cells are usually disposed of by cell death.

Underlying the remarkable flexibility and spatiotemporal control of the ATM-NEMO–IKK–NF-κB system are a series of modifications of the NEMO protein itself, and several other proteins in this cascade (*2*, *5*, *6*) (see the figure). First, NEMO becomes modified by addition of a peptide called SUMO (*10*), in an ATM-independent manner (*5*). The addition of SUMO prevents nuclear exit of NEMO and earmarks it for phosphorylation by ATM (*2*). This, in turn, leads to the addition of another peptide, ubiquitin, at the expense of the SUMO moiety. Ubiquitinated NEMO, together with ATM, then exit the nucleus and activate cytoplasmic IKK. Such hitch-hiking of ATM, with NEMO as vehicle, and the requirement for ATM activity during IKK activation are themselves unprecedented and lend support to the controversial notion that ATM may perform some important roles outside the nucleus as well. There are likely additional protein modifications to be elucidated in this cascade, and overall, this phenomenon highlights one of the emerging concepts in contemporary biology: Diverse protein modifications greatly enhance the versatility of cellular processes such as signal transduction, subcellular trafficking, and protein interactions, and so on.



ATM finds NEMO in response to DNA damage. Genotoxic insults that cause double-strand breaks in DNA evoke a "stress" signal that promotes SUMO modification of nuclear NEMO (*5*, *6*), and prevents its nuclear export. In parallel, the nuclear ATM kinase (*1*) becomes activated by signals generated upon double-strand breaks. SUMO-modified NEMO is phosphorylated by active ATM, and this leads to subsequent removal of SUMO and attachment of ubiquitin (Ubi) to NEMO (*2*). Modified NEMO that is associated with ATM exits the nucleus and then associates with, and activates, the IKK complex (*2*). IKK activation requires the ELKS protein and active ATM kinase (*2*, *7*). IKK then activates NF-κB, which undergoes nuclear translocation and switches on transcription of prosurvival genes.

The delicate balance in the ATM-NEMO–IKK–NF-κB system also has, unfortunately, a dark side. Too little or too much activation of this pathway may result in abnormal cells and threaten the organism. Indeed, aberrations in several components of the cascade do occur in humans, and cause diseases due to inappropriate immune responses, inflammatory reactions, or an imbalance between cell proliferation and cell death (*4*, *11*). Evidence is accumulating that, due to cancer-associated abnormalities in the incoming signaling pathways or the IKK–NF-κB system itself, the prosurvival activity of NF-κB is often activated constitutively in diverse types of cancer (*11*). This makes such tumors highly resistant to radiation and chemotherapy (*11*). In addition, given the recently discovered role of the DNA damage response pathways in guarding against progression of premalignant lesions (*12*, *13*), the aberrant constitutive activation of NF-κB may enhance the survival of the incipient cancer cells, rather than eliminate the premalignant cells by enforcing cell death. Hence, deregulated activation of NF-κB may promote the emergence and progression of tumors.

The Wu *et al*. study (*2*) and other recent work (*4*–*7*) raise a host of intriguing questions. It would be important to identify the enzymes responsible for the SUMO and ubiquitin modifications of NEMO. Also, the molecular basis of the ATM-independent stress signal that leads to sumoylation of NEMO remains unknown, as does the precise role of ATM in the cytoplasmic activation of IKK and the nature and roles of additional protein modifications in this pathway. DNA damage and the NF-κB activation mechanisms are intimately linked with diverse pathological states and clinical responses to anticancer treatment. Current efforts to identify agents to block or modulate these processes (*3*, *14*, *15*) may provide not only valuable research tools but also potent drugs to fight major human diseases.

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SCIENCE AND SECURITY

Richard Garwin, at AAAS Event, Details the Dangers of Proliferation

Richard Garwin has been an inventor and a scholar, an adviser to presidents, and a globe-trotting science-diplomat, but all of his varied works and accomplishments can be summarized in one label: problem-solver.

Garwin has been one of the world's most influential scientists in the post–World War II era, and in a recent appearance at AAAS, his insights ranged across decades and issues. He described his work as an architect of the hydrogen bomb and his continuing efforts to check nuclear proliferation, and assessed a number of global security challenges.

Richard Garwin

Through it all, the 77-year-old physicist displayed the acute grasp of detail and the plain-spoken common sense valued by U.S. leaders since President Dwight Eisenhower—even when he tells them what they don't want to hear.

Garwin struck the most sobering note of the night with a warning that terrorists could obtain a nuclear bomb and target the United States.

"I think there's a 50 percent probability that we'll have such a nuclear explosion [in the United States] in the next 4 or 5 years," he said. "We ought to be doing what we can to prevent it. And we ought to be doing what we need to do to keep the damage that that causes localized, rather than destroying the whole society because of a foolish concentration of fundamental elements in a particular location."

Garwin answered questions posed by David Kestenbaum, a science correspondent for National Public Radio, before a packed auditorium at AAAS headquarters in Washington, D.C. The 10 January event was organized by the AAAS Center for Science, Technology, and Security Policy, which seeks to advance the integration of science and public policy for national and international security.

Center Director Norman Neureiter introduced Garwin, calling him "the quintessential example of a scientist who has spent his life in the service of the national security interests of the United States and who has brought his own particular genius to an incredible range of challenges related to security."

Since obtaining his Ph.D. from the University of Chicago in 1949, Garwin has authored more than 500 papers, coauthored seven books, and received 45 patents in fields ranging from laser printing and mass data storage systems, to integrated circuit technology—and mussel-washing. (He and a friend, chemist Harold Friedman, invented a device to clean sand from the black-shelled morsels they gathered from the waters near Friedman's Long Island home.) He won the National Medal of Science in 2003.

Garwin was 23 and working at Los Alamos Scientific National Laboratory when Edward Teller told him about the secret invention of "radiation implosion." Teller asked him to devise an experiment to demonstrate the principle. Garwin returned with a detailed sketch of an 80-ton device; the device was developed under the code-name "Mike" and then detonated in 1952 as the world's first hydrogen bomb.

Garwin said he was emotionally unaffected by the H-bomb's power and significance. Did he see the test explosion? "I haven't seen any nuclear explosions," he told Kestenbaum. "I hope never to see it. I don't need to—I have a good imagination."

While much of his career—including almost 41 years at IBM—has focused on military technology, nuclear nonproliferation has been central to his work. At the AAAS event, he suggested the United States, Russia, and other powers could reduce nuclear stockpiles from tens of thousands of bombs to a few hundred.

When asked what counsel he would give on the charged nuclear negotiations with North Korea and Iran, he said these are serious problems and that the U.S. role in enforcing nations' obligations has been weakened by too often playing close to the margin. U.S. leaders should have taken the advice of various commissions beginning in 1999 to work more aggressively to secure nuclear materials in Russia, Pakistan, and elsewhere, he said.

The United States should work closely with Pakistan to assure its nuclear materials are secure, Garwin said. Iran has "every right" to civilian nuclear technology, he added. But if it has been violating its obligations under the Nuclear Non-Proliferation Treaty, that may warrant United Nations sanctions and "may require empowering indipvidual countries to take military action."

[For a video of Garwin's presentation, see www.aaas.org/news/garwin/]

AAAS AWARDS

U.S., Russian Scientists Win Cooperation Award

A team of seven scientists—three from Russia and four from the United States—has won the 2005 AAAS International Scientific Cooperation Award for their pioneering work to catalog satellites and other objects floating in space. Their collaboration, begun in 1994, has been crucial in improving satellite performance and helping assure the safety of human space missions.

At the beginning of the Space Age, the United States and the former Soviet Union created separate systems for surveying space; they classified the objects floating in space and detailed their orbits. After the fall of the Soviet Union, the scientists overcame decades of mistrust and lingering bureaucratic obstacles to hold a series of workshops, exchanging data on their space surveillance systems and, eventually, comparing their space object catalogs.

The winners: Kyle T. Alfriend, Texas A&M University; Paul J. Cefola, a consultant and lecturer at the Massachusetts Institute of Technology; Felix R. Hoots, AT&T; Andrey I. Nazarenko, Russian Aviation-Space Agency; P. Kenneth Seidelmann, University of Virginia; Stanislav S. Veniaminov, Russian Department of Defense; and Vasiliy S. Yurasov, Space Informatics Analytical Systems (KIA Systems) in Moscow.

The award was presented 18 February at the AAAS Annual Meeting in St. Louis, Missouri. For more information on this and other honors awarded at the meeting, see www.aaas.org/aboutaaas/awards/.

—Barbara Rice contributed to this report.

Bacterial Small-Molecule Signaling Pathways

Andrew Camilli[1,2] and Bonnie L. Bassler[1,3]*

Bacteria use diverse small molecules for extra- and intracellular signaling. They scan small-molecule mixtures to access information about both their extracellular environment and their intracellular physiological status, and based on this information, they continuously interpret their circumstances and react rapidly to changes. Bacteria must integrate extra- and intracellular signaling information to mount appropriate responses to changes in their environment. We review recent research into two fundamental bacterial small-molecule signaling pathways: extracellular quorum-sensing signaling and intracellular cyclic dinucleotide signaling. We suggest how these two pathways may converge to control complex processes including multicellularity, biofilm formation, and virulence. We also outline new questions that have arisen from recent studies in these fields.

One major role of bacterial extracellular small-molecule signaling is in cell-cell communication (quorum sensing), which involves the production, release, and community-wide detection of molecules called autoinducers (*1*). Quorum sensing provides a mechanism for bacteria to monitor one another's presence and to modulate gene expression in response to changes in population density. In the simplest scenario, accumulation of a threshold autoinducer concentration, which is correlated with increasing population density, initiates a signal transduction cascade that culminates in a population-wide alteration in gene expression. The synchronous response of bacterial populations to autoinducers confers a form of multicellularity to bacteria. Hence, many quorum sensing–controlled processes (e.g., bioluminescence, biofilm formation, virulence factor expression, antibiotic production, sporulation, and competence for DNA uptake) require the concerted action of numerous cells to be productive.

Two predominant types of small-molecule autoinducers, acyl homoserine lactones (AHLs) (*2*) and modified oligopeptides (*3*), are used by Gram-negative and Gram-positive bacteria, respectively (Fig. 1). AHLs are synthesized from *S*-adenosyl methionine (SAM) and particular fatty acyl carrier proteins by LuxI-type AHL synthases (*4*). AHL autoinducers all share the core homoserine lactone moiety, but distinct acyl side chains are incorporated into the signal molecules by the various LuxI-type enzymes (Fig. 1). Many AHLs cross membranes freely and are detected in the cytoplasm by LuxR-type proteins. Upon ligand binding, the LuxR-AHL complexes bind DNA promoter elements and activate transcription of quorum sensing–controlled genes (*2*). The specificity of the LuxR-AHL interaction is conferred by an acyl binding pocket in the LuxR protein, which precisely accommodates the acyl chain of its cognate AHL signal (*5*).

Gram-positive bacterial oligopeptide autoinducers range from 5 to 17 amino acids in length (Fig. 1) and are often posttranslationally modified by the incorporation of lactone and thiolactone rings, lanthionines, and isoprenyl groups. Oligopeptide autoinducers are detected by membrane-bound two-component signaling proteins, and signal transduction occurs by a phosphorylation cascade (*6*). Like AHLs, different oligopeptide autoinducers often contain subtle variations, which confer signaling specificity because of the discriminatory properties of their cognate receptors. Some bacteria release and detect multiple AHLs or multiple oligopeptides that control distinct sets of target genes (*1*).

These categories of signals are not comprehensive because several other small-molecule quorum-sensing autoinducers have recently been discovered. Among these, two discoveries (PQS and AI-2) are especially interesting.

The first, 2-heptyl-3-hydroxy-4-quinolone (PQS, for *Pseudomonas* quinolone signal) (Fig. 1) (*7*, *8*), is produced by the opportunistic pathogen *Pseudomonas aeruginosa*, a colonizer of the lungs of people with cystic fibrosis (CF) (*9*). These infections, in which the bacteria are presumed to exist in biofilms, can persist for decades, are recalcitrant to antibiotic treatment, and are a major cause of mortality in CF patients. Together with two well-studied AHL autoinducers, PQS functions as a quorum-sensing signal to control a battery of genes required for virulence factor expression and biofilm formation (*10*, *11*). PQS is quite hydrophobic, obscuring any obvious mechanism for it to act as an extracellular signal; however, an exciting new study shows that a specialized vesicular transport mechanism conveys the PQS signal between *P. aeruginosa* cells (*12*). The PQS signal and other quinolones/quinolines are packaged into endogenously produced membrane vesicles that traffic the molecules between the bacterial cells. The vesicles are proposed to be crucial for efficient information transfer between *P. aeruginosa* cells existing in biofilms in CF sputum. Consistent with this mechanism, mutants that do not produce the vesicles do not exhibit quorum sensing–mediated communication.

P. aeruginosa produces 55 quinolones/quinolines, and although the initial steps in their biosynthesis are identical, the terminal steps are unique to each entity. For example, in the case of PQS, the product of *pqsH* catalyzes the final biosynthetic step. Membrane vesicle formation does not occur in a *P. aeruginosa pqsH* mutant even though the other 54 quinolones/quinolines are still produced. Addition of exogenous PQS restores vesicle formation to the *pqsH* mutant, and surprisingly, also to a *pqsA* mutant that is defective in production of all quinolones/quinolines. Together these experiments suggest that PQS is the critical quinolone both for signaling and for vesicle formation (*12*).

The *P. aeruginosa* membrane vesicles fuse with recipient cells, and their cargo is delivered internally, so it seems that the membrane vesicles protect the quinolones/quinolines from degradation in the environment and may also facilitate mass delivery of these molecules to neighboring cells. Additionally, many of the *P. aeruginosa* quinolones/quinolines have antibiotic activity against Gram-positive cells (*8*), so when the vesicles are delivered to a competing bacterial species, this mode of trafficking and internal delivery of contents could boost the antibacterial efficacy of quinolones/quinolines.

The second autoinducer that we highlight is AI-2. It is produced and detected by a wide variety of bacteria and is proposed to enable interspecies communication (*1*). The AI-2 synthases, called LuxS, all produce the molecule 4,5-dihydroxy-2,3-pentanedione (DPD), which undergoes a variety of spontaneous rearrangements (*13*). Different species of bacteria recognize distinctly rearranged DPD moieties (Fig. 2), which allows bacteria to respond to AI-2 derived from their own DPD and also to that produced by other bacterial species (*13*, *14*). Some bacteria, including *Escherichia coli* and *Salmonella enterica* serovar Typhimurium, produce and consume AI-2 (*15*). Examination of gene expression in mixtures of different species of bacteria shows that when *E. coli* produces AI-2, nearby bacterial species initiate quorum sensing–controlled behaviors in response to cumulative cell number. By contrast, consumption of AI-2 by *E. coli* causes neighboring species to underestimate population density, and hence they fail to initiate or incorrectly terminate quorum sensing (*16*). Pro- and anti-AI-2–mediated interactions could occur in natural niches, and

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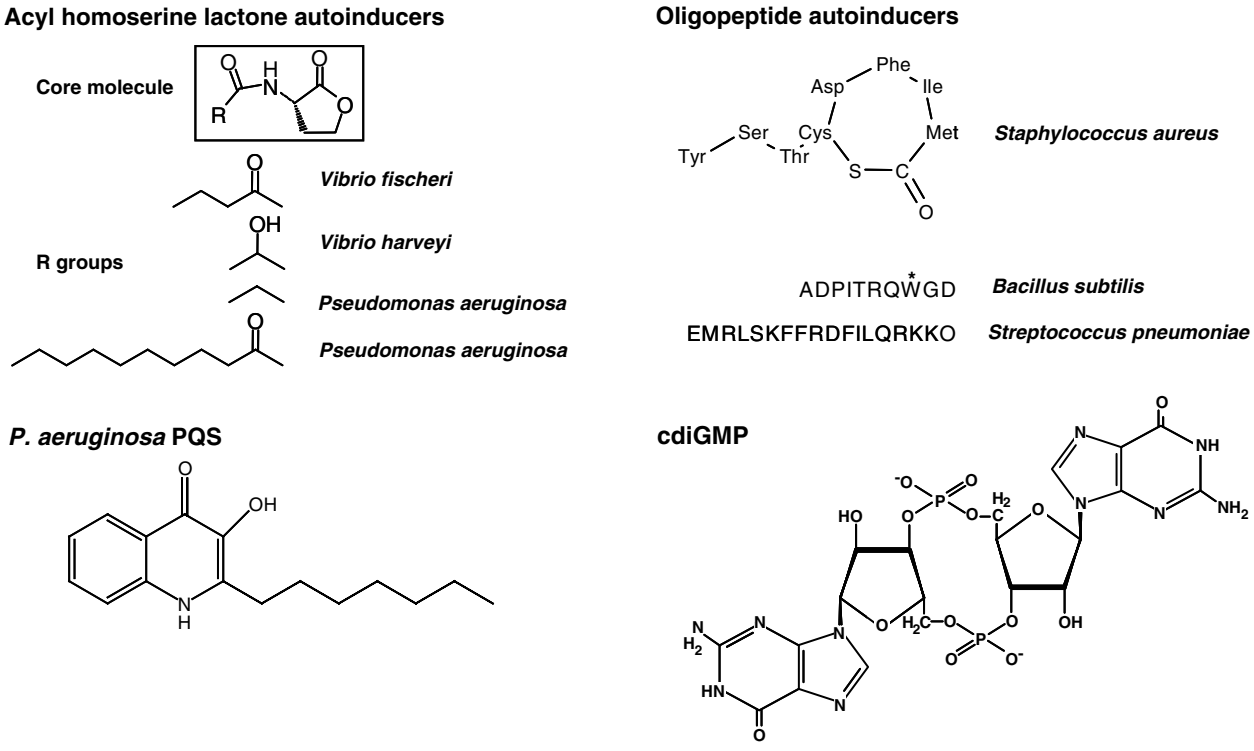

Fig. 1. Small-molecule bacterial signals. Representative structures of autoinducer molecules used in bacterial cell-cell communication, and of the intracellular signaling molecule cdiGMP. The asterisk on the tryptophan residue of the *Bacillus subtilis* oligopeptide autoinducer represents an isoprenyl modification.

furthermore, eukaryotes could profit from these signaling manipulations by evolving particular associations with bacterial species that use or interfere with AI-2–mediated communication. Such associations may be important for the maintenance of the normal human gut microflora and in bacterial disease.

Other prokaryote-prokaryote and eukaryote-prokaryote mechanisms for interference with AHL and oligopeptide signaling have been reported. For example, different strains of *Staphylococcus aureus* produce similar oligopeptide autoinducers that stimulate their own quorum-sensing cascades while cross-inhibiting oligopeptide-mediated signaling in other strains (*17*). Many *Bacillus* species release an enzyme, AiiA, that cleaves the lactone rings from AHLs, rendering them impotent (*18*). The alga *Delisea pulchra* coats its surface with a mixture of halogenated furanones that are structurally similar to AHLs. The furanones are internalized by bacteria, bind to LuxR-type proteins, and destabilize them (*19*). Primary and immortalized human epithelial cell lines inactivate a *P. aeruginos*a AHL autoinducer, suggesting that humans may have evolved quorum-sensing interference strategies for resisting pathogens (*20*). These natural quorum-sensing interference strategies have been exploited in a number of systems to inhibit bacteria that depend on quorum sensing for virulence. Analogous mechanisms for enhancing quorum sensing–controlled behaviors probably also exist and may play out in niches in which such behaviors benefit the organisms cohabitating with quorum-sensing bacteria.

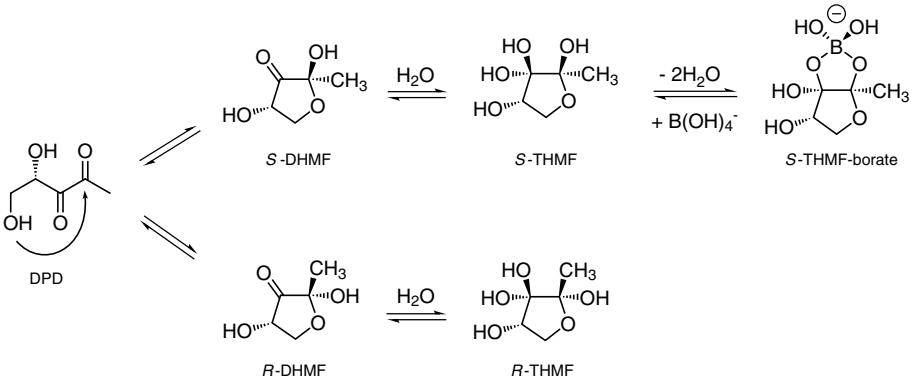

Fig. 2. AI-2: an interconverting family of extracellular signal molecules. The precursor molecule, DPD, undergoes various rearrangements and additional reactions to form distinct biologically active AI-2 signal molecules. The *Vibrio harveyi* AI-2 (*S*-THMF-borate) is produced by the upper pathway, and the *Salmonella enterica* serovar Typhimurium AI-2 (*R*-THMF) is produced by the lower pathway (*13*, *14*).

Intracellular Small-Molecule Signaling

How are population-wide responses to small molecules related to the responses of individual bacteria? Decoding extracellular information requires signal transduction across the bacterial cell membrane. For quorum-sensing signals, this is mediated either by diffusion of autoinducers across the membrane or by phosphorelay from membrane-bound receptors feeding into cytoplasmic second-messenger cascades. Second-messenger systems can integrate many sensory inputs and offer flexibility of recognition and response.

Adenosine 3′,5′ monophosphate (cAMP) and guanosine-3,5-bis(pyrophosphate) (ppGpp) are common second messengers in bacteria. cAMP is synthesized from ATP by one or more adenylate cyclases, and it allosterically activates a transcription factor, catabolite regulation protein (CRP), to regulate catabolic operons for use of alternative carbon sources and other cellular processes (*21*). ppGpp is produced from guanosine 5′-triphosphate (GTP) by a ribosome-

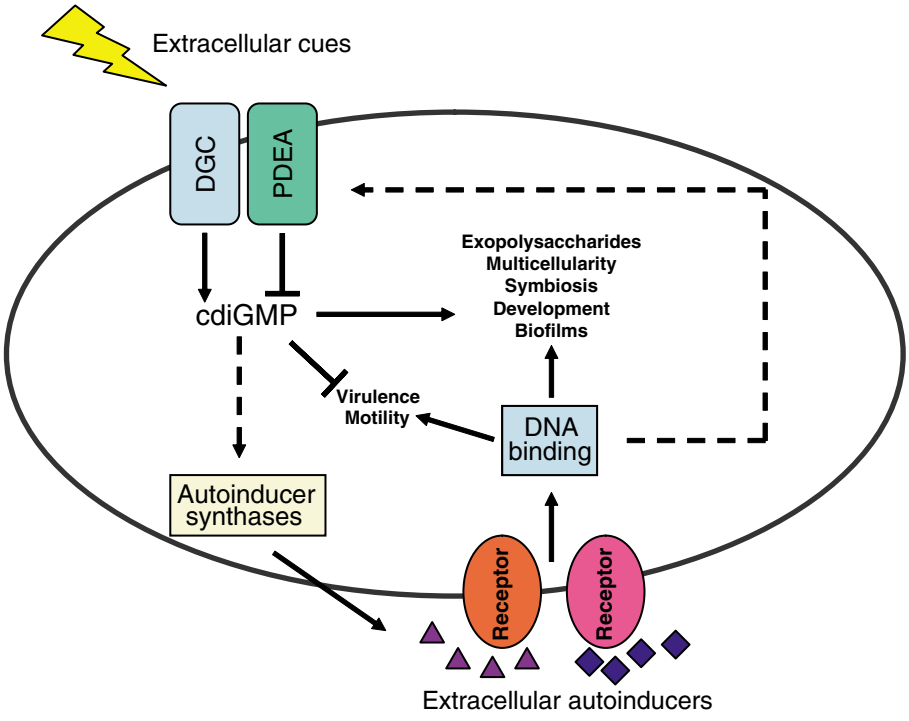

Fig. 3. Possible convergence of quorum sensing and cdiGMP signaling in the regulation of diverse bacterial behaviors. Cell density–dependent extracellular autoinducers activate membrane-bound (shown) or cytoplasmic sensory receptor proteins (not shown) which, through DNA binding proteins, regulate cellular processes including those listed. A variety of extracellular signals activate membrane-bound (shown) or cytoplasmic DGC and PDEA domain proteins (not shown), which synthesize and hydrolyze cdiGMP, respectively. cdiGMP functions as an activator or repressor of many of the same cellular processes regulated by quorum signaling (listed). Hypothetical connections between quorum-sensing and cdiGMP signaling are denoted by dashed lines.

associated protein in response to low levels of charged tRNAs. ppGpp binds to RNA polymerase and alters its activity to repress genes encoding ribosomal RNA and tRNA (*22*), whereas genes specifying amino acid synthesis and transport are activated (*23*). Guanosine 3′,5′-monophosphate (cGMP), an important second messenger in eukaryotes, appears to be rarely used in bacteria. Rather, new evidence suggests that the cyclic dinucleotide 3′,5′-cyclic diguanylic acid (cdiGMP) (Fig. 1) is widely used by bacteria. We concentrate on this molecule because recent research indicates that it serves as the focal point for several extracellular sensory inputs, and because of its role in regulating complex cellular processes that are also regulated by quorum sensing.

cdiGMP was first identified as an allosteric activator of cellulose synthase in *Gluconacetobacter* (formerly *Acetobacter*) *xylinum* (*24*), a bacterium associated with grapes. Diguanylate cyclases (DGCs) and phosphodiesterases A (PDEAs) are responsible for synthesis and breakdown of cdiGMP, respectively (*25*–*27*). The best studied DGC is PleD, one of several signaling proteins required for cellular differentiation in the aquatic bacterium *Caulobacter crescentus*. Analysis of the crystal structure of PleD complexed with cdiGMP led to a model in which two PleD monomers dimerize and catalyze cdiGMP synthesis from two molecules of GTP (*28*). Phosphorylation of PleD regulates its DGC activity, presumably by promoting dimerization (*25*). Regulation of DGC activity by protein phosphorylation also occurs in *Borrelia burgdorferi*, the causative agent of Lyme disease (*26*). Thus, phosphorelays can influence second-messenger cdiGMP pathways. It is not known if other signaling systems, such as those involving cAMP or ppGpp, intersect with cdiGMP pathways.

DGC and PDEA domain proteins are found in most bacterial phyla but are absent from Archaea and Eukarya (*30*). In some genera, such as *Vibrio* and *Pseudomonas*, these modules exist in many dozens of proteins. Their occurrence in transmembrane or membrane-associated proteins that contain sensory domains led to the prediction that DGC and PDEA domains were important in relaying external sensory information into the cytoplasm. Environmental stimuli, such as molecular oxygen, amino acids, electrons, and photons, are believed to regulate the activity of DGC or PDEA proteins (*29*, *30*), and it seems that cdiGMP is the common second messenger for many external signals.

It is clear that one regulatory function mediated by cdiGMP is the control of gene expression. For example, early during cholera disease, the internal cdiGMP concentration in *Vibrio cholerae* is reduced, leading to activation of virulence genes and repression of biofilm-formation genes (*31*, *32*). Similarly, reduction of cdiGMP in *Salmonella enterica* serovar Typhimurium activates the expression of virulence genes required for survival within host cells (*33*). Despite rapid advances in our understanding of cdiGMP as a signaling molecule, we have yet to discover the molecular mechanism underlying its regulatory effects. It may influence DNA binding proteins and thus directly affect gene expression, and/or act on structural proteins and enzymes and direct cell physiology through posttranscriptional mechanisms.

One model for cdiGMP signaling is that it rapidly diffuses throughout the cytoplasm to act as a common allosteric regulator of proteins that control various processes. A contrasting model is that cdiGMP is spatially restricted to microdomains near the cytoplasmic membrane, where fluxes in its concentration mediate allosteric regulation of nearby membrane-bound or membrane-associated proteins. This would allow the cell to have distinct cdiGMP-regulated responses to different stimuli. Consistent with this second model, in *G. xylinus*, virtually all DGC and PDEA proteins, 90% of the total cellular cdiGMP, and the only known target of cdiGMP regulation (cellulose synthase) are located in the membrane fraction (*27*, *34*). Additionally, PleD becomes localized to one cell pole in *C. crescentus* following phosphorylation and activation by its cognate sensor kinases, suggesting that a localized flux of cdiGMP may be important for subsequent developmental changes in this organism (*26*). A somewhat analogous situation exists in eukaryotic cells where diffusion of cAMP appears, in some cases, to be restricted to cellular microdomains by the organization of cAMP-specific phosphodiesterases into a boundary (*35*, *36*).

The preponderance of proteins containing DGC and PDEA domains in some species of bacteria (e.g., 61 in *V. cholerae*) lends credence to the concept that spatial restriction of cdiGMP provides high-fidelity signaling. However, recent data suggest that the enzymatic activities of most DGC and PDEA proteins are tightly regulated by phosphorylation or other modifications (*25*). Thus, the background activities of DGC and PDEA enzymes could be minimal under most conditions, so spatial restriction of cdiGMP may not be a general requirement. Identifying the subcellular locations of and protein-protein interactions between bacterial DGC, PDEA, and cdiGMP-regulated proteins will help to distinguish whether one or the other model, or both models, operate.

Quorum sensing and cdiGMP signaling regulate some of the same complex processes, namely biofilm formation, multicellularity, and virulence, so it stands to reason that these two signaling pathways may be linked. Although a direct connection, such as an autoinducer activating a membrane-bound DGC or PDEA, has

yet to be reported, the evidence for indirect interplay is strong. In *V. cholerae*, the quorum sensing–regulated transcription factor AphA influences expression of genes encoding DGCs and PDEAs in addition to those encoding virulence factors (*37*). Also, formation of a symbiotic biofilm community between the hyperthermophiles *Thermotoga maritima* and *Methanococcus jannaschii* is mediated by quorum sensing and, very likely, cdiGMP signaling, because genes for DGCs and PDEAs were up-regulated and down-regulated, respectively, during formation of the biofilm (*38*).

Conclusions and Prospects

In our opinion, future research will reveal an explicit connection between extracellular quorum-sensing signaling and intracellular cdiGMP signaling. That quorum sensing and cdiGMP play critical roles in biofilm formation, as well as in other related processes, is a clue that the two signaling processes converge. Many non–mutually exclusive possibilities exist for interconnections between quorum sensing and cdiGMP signaling in different bacteria (Fig. 3). As in *T. maritima*, quorum sensing could regulate the expression of genes encoding proteins with DGC and PDEA activities, and thus, quorum sensing controls the cellular level of cdiGMP. Alternatively or additionally, cdiGMP could impinge on the expression or the activity of autoinducer synthases and thus affect production of quorum-sensing signals. Feedback regulation between the two kinds of signaling processes might also exist. Ultimately, if a molecular link is discovered between quorum sensing and cdiGMP signaling, it will confirm that bacteria can convert extracellular population-density information into intracellular second-messenger signals that change gene expression, cellular physiology, and group behavior. It is very likely that similar molecular signaling mechanisms underpin collective behaviors of cells in higher organisms.

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Prions in Skeletal Muscles of Deer with Chronic Wasting Disease

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Prions are transmissible proteinaceous agents of mammals that cause fatal neurodegenerative diseases of the central nervous system (CNS). The presence of infectivity in skeletal muscle of experimentally infected mice raised the possibility that dietary exposure to prions might occur through meat consumption (*1*). Chronic wasting disease (CWD), an enigmatic and contagious prion disease of North American cervids, is of particular concern. The emergence of CWD in an increasingly wide geographic area and the interspecies transmission of bovine spongiform encephalopathy (BSE) to humans as variant Creutzfeldt Jakob disease (vCJD) have raised concerns about zoonotic transmission of CWD.

To test whether skeletal muscle of diseased cervids contained prion infectivity, Tg(CerPrP) mice (*2*) expressing cervid prion protein (CerPrP) were inoculated intracerebrally with extracts prepared from the semitendinosus/semimembranosus muscle group of CWD-affected mule deer or from CWD-negative deer. The availability of CNS materials also allowed for direct comparisons of prion infectivity in skeletal muscle and brain. All skeletal muscle extracts from CWD-affected deer induced progressive neurological dysfunction in Tg(CerPrP) mice, with mean incubation times ranging between 360 and ~490 days, whereas the incubation times of prions from the CNS ranged from ~230 to 280 days (Table 1). For each inoculation group, the diagnosis of prion disease was confirmed by the presence of disease-associated, protease-resistant PrP (PrP^{Sc}) in the brains of multiple infected Tg(CerPrP) mice [see (*3*) for examples]. In contrast, skeletal muscle and brain material from CWD-negative deer failed to induce disease in Tg(CerPrP) mice (Table 1), and PrP^{Sc} was not detected in the brains of asymptomatic mice as late as 523 days after inoculation (*3*).

Our results show that skeletal muscle as well as CNS tissue of deer with CWD contains infectious prions. Similar analyses of skeletal muscle from BSE-affected cattle did not reveal high levels of prion infectivity (*4*). It will be important to assess the cellular location of PrP^{Sc} in muscle. Although PrP^{Sc} has been detected in muscles of scrapie-affected sheep (*5*), previous studies failed to detect PrP^{Sc} by immunohistochemical analysis of skeletal muscle from deer with natural or experimental CWD (*6*, *7*). Because the time of disease onset is inversely proportional to prion dose (*8*), the longer incubation times of prions from skeletal muscle extracts compared with those from matched brain samples indicated that prion titers were lower in muscle than in the CNS, where infectivity titers are known to reach high levels. Although possible effects of CWD strains or strain mixtures on these incubation times cannot be excluded, the variable 360- to ~490-day incubation times suggested a range of prion titers in skeletal muscles of CWD-affected deer. Muscle prion titers at the high end of the range produced the fastest incubation times, which were ~30% longer than the incubation times of prions from the CNS of the same animal. Because all mice in each inoculation group developed disease, prion titers in muscle samples producing the longest incubation times were higher than the end point of the bioassay, defined as the infectious dose at which half the inoculated mice develop disease. Although the risk of exposure to CWD infectivity after consumption of prions in muscle is mitigated by relatively inefficient prion transmission via the oral route (*9*), our results show that semitendinosus/semimembranosus muscle, which is likely to be consumed by humans, is a major source of prion infectivity. Humans consuming or handling meat from CWD-infected deer are therefore at risk to prion exposure.

Table 1. Incubation times after inoculation of Tg(CerPrP) mice with prions from skeletal muscle and brain samples of CWD-affected deer. PBS, phosphate buffered saline.

Inocula	Incubation time, mean days ± SEM (n/n_0)*	
	Skeletal muscle	Brain
	CWD-affected deer	
H92	360 ± 2 (6/6)	283 ± 7 (6/6)
33968	367 ± 9 (8/8)	278 ± 11 (6/6)
5941	427 ± 18 (7/7)	
D10	483 ± 8 (8/8)	231 ± 17 (7/7)
D08	492 ± 4 (7/7)	
Averages	426	264
	Nondiseased deer	
FPS 6.98	>523 (0/6)	
FPS 9.98	>454 (0/7)	>454 (0/6)
None	>490 (0/6)	
PBS	>589 (0/5)	

*The number of mice developing prion disease (n) divided by the original number of inoculated mice (n_0) is shown in parentheses. Mice dying of intercurrent illnesses were excluded.

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Supporting Online Material

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Fig. S1

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Noncoding RNAs of Trithorax Response Elements Recruit *Drosophila* Ash1 to Ultrabithorax

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Homeotic genes contain cis-regulatory trithorax response elements (TREs) that are targeted by epigenetic activators and transcribed in a tissue-specific manner. We show that the transcripts of three TREs located in the *Drosophila* homeotic gene *Ultrabithorax* (*Ubx*) mediate transcription activation by recruiting the epigenetic regulator Ash1 to the template TREs. TRE transcription coincides with *Ubx* transcription and recruitment of Ash1 to TREs in *Drosophila*. The SET domain of Ash1 binds all three TRE transcripts, with each TRE transcript hybridizing with and recruiting Ash1 only to the corresponding TRE in chromatin. Transgenic transcription of TRE transcripts restores recruitment of Ash1 to *Ubx* TREs and restores *Ubx* expression in *Drosophila* cells and tissues that lack endogenous TRE transcripts. Small interfering RNA–induced degradation of TRE transcripts attenuates Ash1 recruitment to TREs and *Ubx* expression, which suggests that noncoding TRE transcripts play an important role in epigenetic activation of gene expression.

The identity of cells in metazoan organisms is established during development and mitotically propagated throughout the entire life cycle. Phylogenetically highly conserved protein families of epigenetic regulators determine the fate of developing cells by establishing and maintaining mitotically stable gene expression programs (*1*–*4*). In *Drosophila*, members of the trithorax group (trxG) of epigenetic regulators maintain active transcription states, whereas members of the Polycomb group (PcG) maintain repressed transcription states (*2*–*4*). Many epigenetic regulators control gene expression by establishing transcriptional competent or silent chromatin structures (*5*, *6*). Several epigenetic activators [Trx, trithorax-related (Trr)] and repressors (Enhancer of zeste) are lysine-specific histone methyltransferases (HMTs) and contain a SET domain, the catalytic hallmark motif of HMTs. Methylation of lysine residues in histones H3 and H4 has been correlated with epigenetic activation [Lys^4 in H3 (H3-K4)] and repression [Lys^9 and Lys^{27} in H3 (H3-K9)] (*6*–*8*).

We previously showed that the epigenetic activator "absent small and homeotic discs" (Ash1) promotes transcriptional activation by trimethylating H3-K4, H3-K9, and Lys^{20} in H4 (H4-K20) (*9*). Ash1 maintains activated transcription states in larval imaginal discs that give rise to the appendages in the adult fly (*10*, *11*). For example, Ash1 is essential for the expression of the homeotic gene *Ultrabithorax* (*Ubx*) in third-leg and haltere imaginal discs, and *Ubx* expression coincides with Ash1-mediated histone methylation (*9*–*11*).

PcG and trxG regulators are recruited to specific chromosomal elements that are present in the cis-regulatory region of target genes (*2*–*4*). The same element can act as an activating or a silencing module (*4*). In the repressed state, the elements represent Polycomb response elements (PREs) and facilitate the recruitment of PcG proteins (*2*–*4*). In the activated state, the DNA-elements function as trithorax response elements (TREs) and recruit trxG proteins (*3*, *4*). Transcription of noncoding RNAs (ncRNAs) from TRE/PRE elements switches silent PREs into TREs, which indicates that TRE/PRE transcription plays an important role in epigenetic activation (*12*–*15*). How transcription of TREs culminates in the recruitment of trxG regulators is unknown. Here, we address the question of how epigenetic regulators without known DNA binding capabilities, such as Ash1 (*16*), recognize and bind target genes in chromatin.

***Ubx* TREs are transcribed in *Drosophila* imaginal discs.** The coincidence of the tissue-specific transcription and trans-regulatory activity patterns of TREs and trxG proteins, respectively, suggests that not only TRE/PRE transcription but also the resulting ncRNAs might play a role in epigenetic activation (*12*–*15*). Here, we analyze the role of ncRNAs transcribed from three *Ubx* TRE/PREs. The *Ubx* locus contains a cluster of three characterized TRE/PREs (TRE1 to TRE3) within the boundaries of the chromosomal memory element (CME) *bxd* that is located 22 kb upstream of the *Ubx* promoter (Fig. 1A) (*17*, *18*).

To correlate the transcriptional activity of *Ubx* with *bxd* transcription in *Drosophila*, we used rapid amplification of cDNA ends (RACE) to detect *bxd* transcripts in third-leg discs. Three capped, polyadenylated *bxd* transcripts transcribed by RNA polymerase II were detected in third-leg and haltere discs (*tre1*, *tre2*, *tre3*) (Fig. 1A) (*19*).

We next used the reverse transcription polymerase chain reaction (RT-PCR) to determine

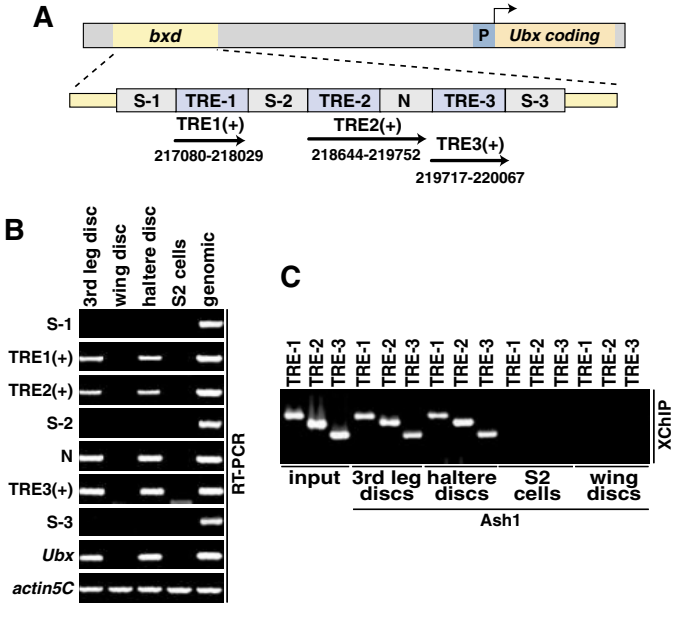

Fig. 1. Cell type–specific transcription of *Ubx* TREs. (**A**) Schematic representation of the *Ubx* locus (top) and the *bxd* DNA element (bottom). The positions of *bxd*, *Ubx* promoter (P), TREs, spacer DNA (S-1, S-2, N, and S-3) are indicated. The orientation and position (*22*) of TRE transcripts in *bxd* are indicated. (**B**) RT-PCR assays were used to detect the transcripts of the indicated *bxd* elements and control transcripts (*actin5C*, *Ubx*) in imaginal discs (third-leg, haltere, and wing), Schneider S2 cells, and genomic DNA. (**C**) PCR analysis of XChIP immunoprecipitates detecting the association of Ash1 with *Ubx* TREs in imaginal discs and S2 cells. Input represents the amount of transcripts or TREs detected in 0.5% of the starting material.

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whether the presence of the three TRE transcripts coincides with *Ubx* transcription. RNA was isolated from third-leg discs and haltere imaginal discs (haltere discs), which both transcribe *Ubx*, and from wing imaginal discs (wing discs) and embryonic *Drosophila* Schneider 2 (S2) cells that do not transcribe *Ubx* (*9*–*11*). Transcripts from *Ubx* and all three TREs were detected in third-leg and haltere discs, whereas *Ubx* and TRE transcripts were not detected in S2 cells and wing discs (Fig. 1B) (figs. S1 and S2).

Recruitment of Ash1 to *Ubx* TREs. To investigate whether Ash1 is recruited to transcriptionally active *Ubx* TREs, we used in vivo cross-linked chromatin immunoprecipitation (XChIP) to detect Ash1 at the *Ubx* TREs in third-leg, haltere, and wing discs and in S2 cells, all of which express *ash1* (*9*, *10*). Ash1 was detected at all three TREs in third-leg and haltere discs (Fig. 1C). In addition, the characteristic Ash1 histone methylation pattern was detectable in all three TREs and the transcriptionally active *Ubx* promoter in third-leg discs (Fig. 1C and Fig. 2A). Ash1 was not detected at the TREs of the transcriptionally inactive *Ubx* locus in wing discs and S2 cells, which do not transcribe TREs (Fig. 1C).

We also compared the recruitment of Ash1 to *Ubx* in wild-type and homozygous mutant $ash1^{22}$ third-leg discs by XChIP. The $ash1^{22}$ mutant is recessive lethal and expresses a truncated protein that lacks the SET domain and trans-activation activity (*10*). Ash1 and the characteristic Ash1 histone methylation pattern were detected at the transcriptionally active *Ubx* locus in wild-type discs but not in $ash1^{22}$ mutant discs (Fig. 2, A and B) (fig. S3); this finding indicates that recruitment of Ash1 and Ash1-mediated histone methylation coincides with activation of *Ubx* expression in third-leg discs. We monitored TRE transcription in the wild-type and $ash1^{22}$ mutant third-leg discs by RT-PCR. TRE transcripts were detected at comparable levels in wild-type and mutant discs, which indicates that Ash1 is not a major regulator of TRE transcription in imaginal discs (Fig. 2C) (fig. S3).

Ash1 SET domain interacts with TRE transcripts in vitro. The association of Ash1 with TREs in cells producing TRE transcripts suggests that TRE transcription or TRE transcripts nucleate recruitment of Ash1 to *Ubx* TREs. SET-domain proteins can bind single-stranded RNA and DNA in vitro, and ncRNA has been implicated in protein recruitment in gene dosage compensation (*20*–*24*). We used in vitro protein-RNA binding assays to assess whether Ash1 associates with TRE transcripts. Ash1SET, which consists of amino acids 1001 to 1619, retained TRE1(+), TRE2(+), and TRE3(+) but not the H3-K9–specific HMT Medusa (Mdu) (Fig. 3A) (fig. S4). In contrast, Ash1, Ash1ΔN, and Mdu did not bind the antisense RNA of the *Ubx* TREs (Fig. 3A) (fig. S4). Ash1ΔN did not interact with the N-element in *tre2* (Fig. 3A), which corresponds to the DNA spacer separating TRE-2 and TRE-3 (Fig. 1A).

In competition experiments, unlabeled TRE transcripts could outcompete the interaction of Ash1 with the corresponding TRE transcript (fig. S5). In contrast, double-stranded TRE transcripts, double-stranded DNA TRE sequences, and DNA-RNA hybrids consisting of TRE transcripts and TREs failed to disrupt the interaction; these findings suggest that Ash1 associates with single-stranded TRE transcripts (fig. S5).

To delineate the RNA-binding motif of Ash1, we investigated the interaction of truncated *ash1* proteins with TRE transcripts. In addition to Ash1SET, we tested Ash1ΔN (amino acids 1001 to 2218), which contains the Ash1 SET module, and Ash1N (amino acids 1 to 1001) and Ash1C (amino acids 1619 to 2218), which both lack the SET domain and cysteine-rich regions (Fig. 3B). Ash1ΔN and Ash1SET, but not Ash1N and Ash1C, retained TRE transcripts, indicating that the SET domain of Ash1 binds TRE transcripts in vitro (Fig. 3C).

RNA-dependent recruitment of Ash1 to *Ubx* TREs in *Drosophila*. We next used XChIP to investigate whether Ash1 associates with TRE transcripts in vivo. Ash1 coprecipitated with TRE transcripts but not control transcripts from mock-treated chromatin (Fig. 3D) (fig. S6). Ash1 bound TRE transcripts in ribonuclease (RNase) III–treated chromatin, indicating that double-stranded RNA (dsRNA) motifs within TRE transcripts do not mediate the association of TRE transcripts with Ash1 in vivo (Fig. 3D) (fig. S7). In contrast, Ash1 did not interact with TRE transcripts from RNase A– and RNase H–treated chromatin, indicating that single-stranded RNA (ssRNA) is important for the association of Ash1 with TRE transcripts (Fig. 3D) (fig. S7). The disruption of the association between Ash1 and TRE transcripts by RNase H (which degrades DNA-RNA hybrids) in chromatin suggests that TRE transcripts hybridize with DNA in chromatin.

Is the association of Ash1 with TREs dependent on RNA? We used XChIP to compare the interaction of Ash1 and TRE in mock- and RNase-treated chromatin. Antibodies to Ash1 precipitated all three TREs, but not the spacer DNAs (S-2), from mock-treated and RNase III–treated chromatin, indicating that dsRNA does not contribute to the interaction of Ash1 with TREs (Fig. 3E) (fig. S7). In contrast, treating chromatin with RNase H or RNase A attenuated the association of Ash1 with TREs, indicating that the association of Ash1 with the *Ubx* TREs is RNA-dependent (Fig. 3E) (fig. S7). The disruption of the interaction of Ash1 with TREs in chromatin by RNase H and RNase A raises the possibility that ssRNA motifs in RNA-DNA hybrids play a role in the recruitment of Ash1 to TREs.

To verify that the observed attenuation of Ash1-TRE interactions is based on specific rather than general disruption of protein-DNA interactions in RNase-treated chromatin, we investigated the recruitment of the general transcription factor TFIID to target genes in mock- and RNase-treated chromatin (*25*). The TATA-binding protein (TBP) subunit of TFIID

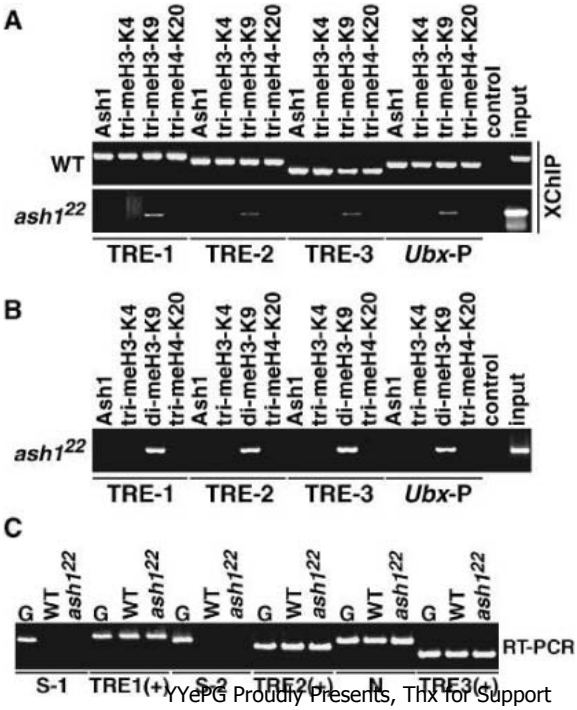

Fig. 2. Recruitment of Ash1 to *Ubx* TREs in third-leg imaginal discs. (**A**) PCR analysis of XChIP immunoprecipitates detecting the association of Ash1 and the Ash1 histone methylation pattern at the TREs and promoter of *Ubx* in wild-type (WT) and $ash1^{22}$ mutant third-leg imaginal discs. In vivo cross-linked chromatin was immunoprecipitated with the indicated antibodies and rat/rabbit antiserum (control). Input represents the amount of TRE-1 detected in 0.5% of the starting material. (**B**) XChIP analysis as described in (A), except that chromatin was immunoprecipitated with an antibody to di-methylated H3-K9. (**C**) RT-PCR analysis detecting *bxd* transcripts in RNA pools isolated from wild-type (WT) and $ash1^{22}$ mutant third-leg discs or in genomic DNA (G).

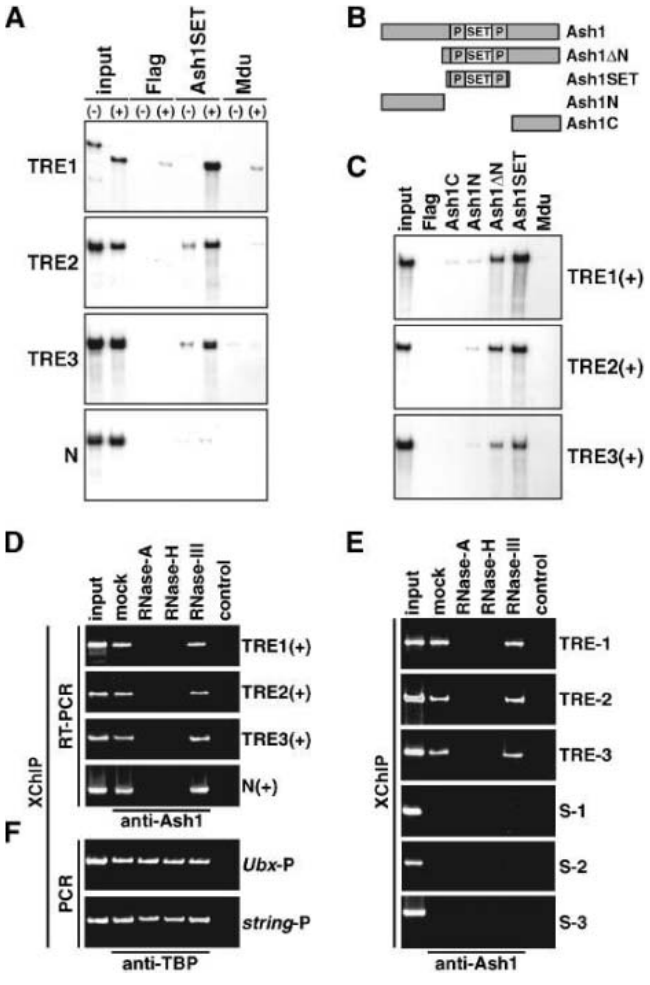

Fig. 3. The SET domain of Ash1 binds TRE transcripts in vitro and in chromatin. (**A**) Autoradiograms of in vitro protein-RNA binding assays (*19*). Radiolabeled sense (+) and antisense (–) transcripts of TRE-1, TRE-2, N, and TRE-3 were incubated with anti-Flag M2 antibody agarose (Flag) or Flag beads loaded with recombinant Ash1SET or Medusa (Mdu). (**B**) Schematic representation of Ash1 and truncated Ash1 derivatives. The position of the SET domain (SET) and pre- and post-SET domains (P) are indicated. (**C**) In vitro protein-RNA binding assays as in (A), except that Flag beads were loaded with Ash1SET, Ash1ΔN, Ash1C, or Ash1N (amino acids 1 to 1001). In (A) and (C), input represents 10% of the input RNA. (**D**) PCR analysis of XChIP immunoprecipitates detecting the association of Ash1 with *bxd* transcripts in mock and RNase-treated and subsequently cross-linked chromatin isolated from third-leg discs. (**E**) XChIP assays were used to detect the association of Ash1 with TREs in chromatin. (**F**) XChIP assays were used to detect the association of TBP to the *Ubx* promoter (*Ubx*-P) and *string/cdc25* promoter (*string*-P) in precipitated DNA pools. In (D) to (F), input represents DNA and RNA detected in 0.5% of the input material.

interacts with the TATA box in eukaryotic promoters (*25*). PCR detected the interaction of TBP with the promoter of *Ubx* and *string*, whose transcription requires TFIID activity (*26*). TBP interacted with both promoters in mock-treated and RNase A–, RNase H–, and RNase III–treated chromatin, indicating that RNase treatment did not attenuate TBP-promoter interactions and protein-gene interactions in general (Fig. 3F) (fig. S7).

To test whether the detected association of Ash1 with TREs and TRE transcripts occurs in chromatin or is the result of fortuitous interactions generated in chemically cross-linked chromatin, we investigated the association of Ash1 with TRE transcripts and TREs in native chromatin with the use of native chromatin immunoprecipitation (NChIP). Ash1 bound all three TREs and TRE transcripts in mock- and RNase III–treated chromatin but not in RNase H– or RNase A–treated chromatin, indicating that Ash1 coimmunoprecipitates with TREs and TRE transcripts in native chromatin (Fig. 4, A and B) (fig. S8). An association of Ash1 with the N portion of the TRE2(+) transcript, as observed in cross-linked chromatin, was not detectable in native chromatin; this result indicates that, as in vitro, Ash1 binds the RNA corresponding to TRE-2 but not the N region of the TRE2(+) transcript.

Collectively, our data indicate that the recruitment of Ash1 to the TREs of *Ubx* is mediated by RNA and suggests the existence of a trimeric protein–nucleic acid complex in chromatin, consisting of Ash1, TREs, and TRE transcripts.

Ash1 is detectable at about 150 loci on *Drosophila* polytene chromosomes (*10*). To assess whether RNA facilitates Ash1 recruitment to target loci other than *Ubx*, we compared the interaction of Ash1 with target loci on

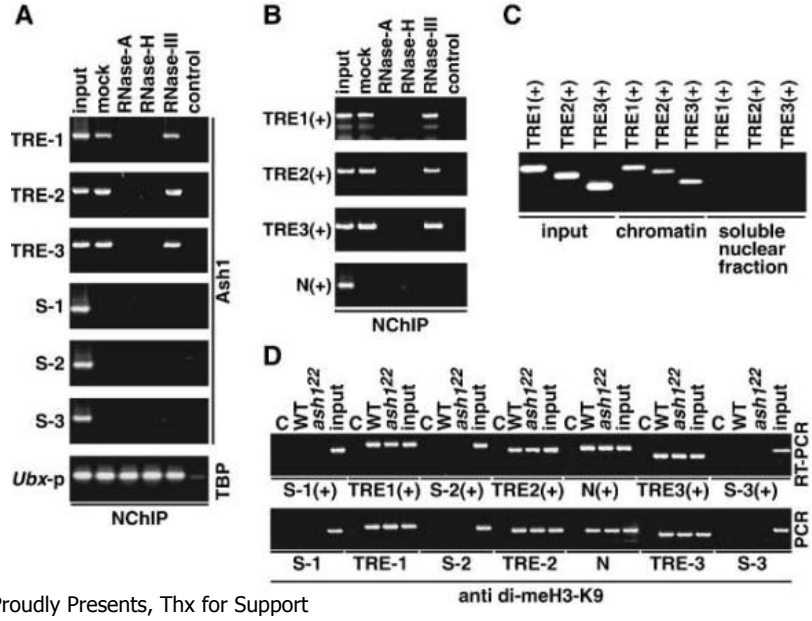

Fig. 4. TRE transcripts mediate the recruitment of Ash1 to *Ubx* TREs in third-leg imaginal discs. (**A**) PCR analysis of NChIP assays detecting the association of Ash1 with *bxd* DNA elements in mock- and RNase-treated chromatin isolated from third-leg imaginal discs. (**B**) RT-PCR analyses of NChIP immunoprecipitates detecting the association of Ash1 with TRE transcripts in native chromatin. (**C**) RT-PCR analyses of NChIP assays detecting the association of Ash1 with TRE transcripts in chromatin and the soluble, histone-free nuclear extract. (**D**) RT-PCR analysis of XChIP RNA immunoprecipitates detecting chromatin-associated *bxd* transcripts (top) and the corresponding *bxd* DNA templates (bottom) in chromatin isolated from wild-type (WT) and *ash1*^{22} mutant third-leg discs. Chromatin was immunoprecipitated with antibodies to dimethylated H3-K9 or rat serum (C). In all panels, input represents the amount of TREs and TRE transcripts detected in 0.5% of the starting material.

mock- and RNase-treated chromosome squashes. Compared to mock-treated chromosomes, RNase treatment attenuated the association of Ash1 with the majority of the target loci (fig. S9). This result suggests that RNA plays an important role in the recruitment of Ash1 to target genes in chromatin.

Ash1 associates with chromatin-bound TRE transcripts. To assess whether TRE transcripts associate with chromatin, we investigated whether Ash1 coprecipitates TRE transcripts from chromatin-free nuclear extract. Ash1 bound TRE transcripts in chromatin but not chromatin-free nuclear extract (Fig. 4C) (fig. S8), indicating that TRE transcripts are preferentially associated with chromatin in the cell.

We used XChIP to determine whether the association of Ash1 with TRE transcripts precedes the recruitment of Ash1 to TREs in chromatin, or vice versa. In vivo cross-linked chromatin was isolated from wild-type and *ash1*^{22} mutant third-leg discs, sheared, and immunoprecipitated with antibodies to dimethylated H3-K9 present at the TREs of the transcriptionally active and inactive *Ubx* locus in third-leg discs (Fig. 2, A and B). The antibody to dimethylated H3-K9 coprecipitated with TREs and TRE transcripts from the chromatin of wild-type and *ash1*^{22} third-leg discs (Fig. 4D) (fig. S10), indicating that TRE transcripts are retained at *Ubx* TREs before recruitment of Ash1.

TRE transcripts recruit Ash1 in trans. To dissect the role of TRE transcripts in *Ubx* transcription, we asked whether transiently transcribed TRE transcripts could restore the recruitment of Ash1 to *Ubx* TREs and *Ubx* expression in S2 cells, which express Ash1 but lack endogenous TRE transcripts. S2 cells were transiently transfected with plasmids transcribing sense or antisense TRE transcripts (*19*) (Fig. 5A) (fig. S11). In PCR assays, *Ubx* transcription was undetectable in S2 cells transiently transcribing antisense TRE transcripts or *mdu* (Fig. 5, A and B). In contrast, *Ubx* transcription was activated by one TRE transcript (Fig. 5B) (fig. S11) and cooperatively activated by multiple TRE transcripts (fig. S12).

We next used XChIP to determine whether activation of *Ubx* transcription by transient TRE transcripts coincides with the recruitment of Ash1 to TREs. In vivo cross-linked chromatin was isolated from wild-type S2 cells and cells transiently transcribing one or multiple TRE transcripts and control RNAs, and it was then immunoprecipitated with antibodies to Ash1 and the Ash1 histone methylation pattern (Fig. 5C). Ash1 was not detected at the TREs of transcriptionally silent *Ubx* in cells transcribing *mdu* or antisense TRE RNAs (Fig. 5C). In contrast, Ash1 and the Ash1 histone methylation pattern were detected at the *Ubx* TREs in cells transcribing TRE1(+), TRE2(+), and/or TRE3(+) (Fig. 5C) (fig. S13). Each of the three TRE transcripts facilitated the association of Ash1 only with the corresponding template TRE but not with other TREs.

To verify the specificity of the described recruitment, we investigated whether TRE transcripts facilitate recruitment of Ash1 to CMEs containing TREs/PREs and genes other than *Ubx*. In XChIP assays, Ash1 was not detected at *Drosophila* genes and the CMEs *MCP* and *Fab7* in S2 cells transcribing TRE1(+), TRE2(+), or TRE3(+) (fig. S14) (*12*, *13*). Thus, TRE transcripts facilitate Ash1 recruitment specifically to the corresponding TRE template DNA.

We used NChIP and XChIP to assess whether transiently transcribed TRE transcripts associate with TREs and Ash1 in chromatin. Native chromatin was isolated from wild-type S2 cells and S2 cells transiently cotranscribing all three sense or antisense TRE transcripts. Ash1 did not associate with TRE transcripts (Fig. 5, D and F) and TREs (Fig. 5, E and G) in cross-linked (Fig. 5, D and E) and native chromatin (Fig. 5, F and G) from S2 cells transcribing *mdu*. In contrast, Ash1 interacted with TREs and TRE transcripts in S2 cells cotranscribing TRE1(+), TRE2(+), and TRE3(+) (Fig. 5, D to G) (fig. S11).

The association of Ash1 with TREs and TRE transcripts was attenuated by RNase A and RNase H but not RNase III (Fig. 5, D to G) (fig. S11). RNase treatment did not abolish the association of TBP with the *Ubx* promoter (Fig. 5, E and G). These results indicate that Ash1 associates with TRE transcripts and TREs in vivo and that TRE transcripts mediate the association of Ash1 with TREs in trans.

To test this hypothesis, we used RNA interference (RNAi) to assess whether degradation of TRE transcripts attenuates recruitment of Ash1 to *Ubx* TREs and *Ubx* expression in third-leg discs (*27*). In vitro cultivated third-leg discs were incubated with small interfering RNAs (siRNAs) targeting all three TRE transcripts or with control siRNA. RT-PCR and XChIP assays indicated that siRNA-mediated degradation of TRE transcripts attenuates *Ubx* transcription and the interaction of Ash1 with TREs (Fig. 6, A and B) (fig. S15).

Next, we used the binary Gal4/UAS system to determine whether ectopic transcription of TRE transcripts restores recruitment of Ash1 to *Ubx* TREs and *Ubx* transcription (*28*). Effector flies carrying a heat-inducible driver (hsp70Gal4) were crossed with reporter flies carrying Gal4-dependent reporter genes (UAS-TRE) consisting of Gal4-responsive UAS DNA sites and a promoter driving the transcription of

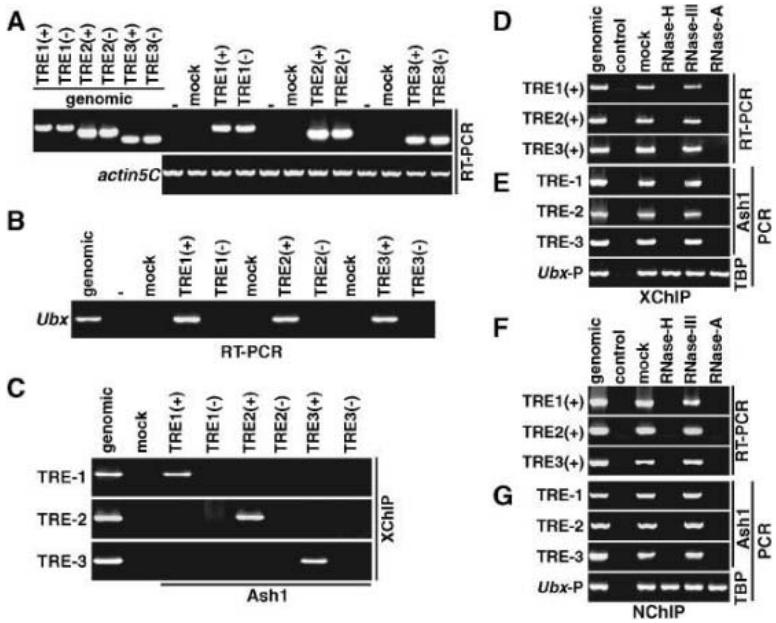

Fig. 5. TRE transcripts reconstitute the interaction of Ash1 with *Ubx* TREs and *Ubx* transcription in S2 cells. (**A**) PCR analysis detecting TRE transcripts and *actin5C* transcription in wild-type S2 cells (–) and S2 cells transfected with plasmids transcribing *mdu* (mock), TRE transcripts [TRE1(+), TRE2(+), TRE3(+)], or antisense TRE transcripts [TRE1(–), TRE2(–), TRE3(–)]. (**B**) PCR assays as in (A) but detecting *Ubx* transcription in wild-type and transfected S2 cells. (**C**) PCR analysis of immunoprecipitates detecting the association of Ash1 with *Ubx* TREs in S2 cells transcribing *mdu* (mock) or sense and antisense TRE transcripts. (**D** and **E**) RT-PCR and PCR analyses of immunoprecipitates detecting the association of Ash1 with *Ubx* TRE transcripts (D) and TREs (E) and TBP with the *Ubx* promoter (*Ubx*-P) (E) in chromatin from S2 cells transiently cotranscribing TRE1(+), TRE2(+), and TRE3(+). (**F** and **G**) RT-PCR (F) and XChIP assays (G) as in (D) and (E), except that native chromatin was used. Transcripts and DNA elements detected in *Drosophila* genomic DNA are also shown.

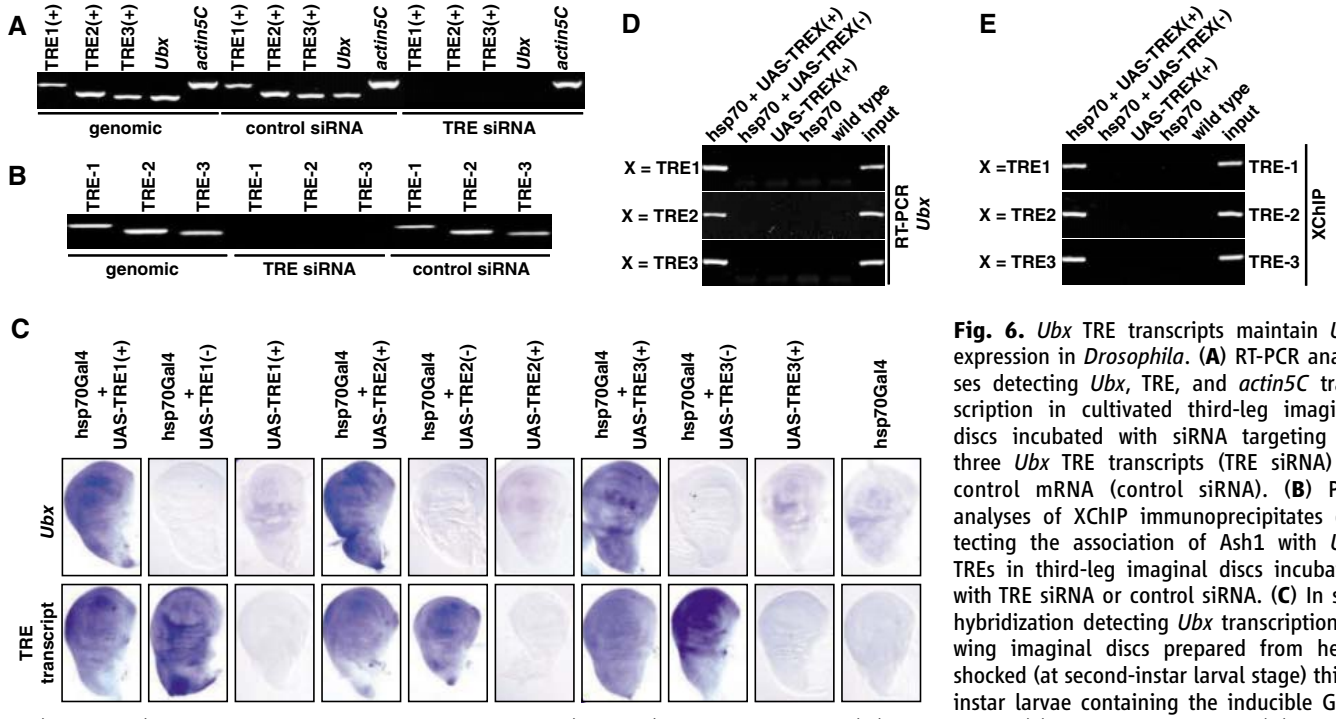

Fig. 6. *Ubx* TRE transcripts maintain *Ubx* expression in *Drosophila*. (**A**) RT-PCR analyses detecting *Ubx*, TRE, and *actin5C* transcription in cultivated third-leg imaginal discs incubated with siRNA targeting all three *Ubx* TRE transcripts (TRE siRNA) or control mRNA (control siRNA). (**B**) PCR analyses of XChIP immunoprecipitates detecting the association of Ash1 with *Ubx* TREs in third-leg imaginal discs incubated with TRE siRNA or control siRNA. (**C**) In situ hybridization detecting *Ubx* transcription in wing imaginal discs prepared from heat-shocked (at second-instar larval stage) third-instar larvae containing the inducible Gal4 driver (hsp70Gal4) and/or Gal4-dependent reporter plasmids (UAS-TRE) transcribing sense (+) or antisense (–) *Ubx* TRE transcripts. (**D**) RT-PCR analyses detecting *Ubx* transcription in wing imaginal discs described in (C). (**E**) PCR analyses of XChIP immunoprecipitates detecting the association of Ash1 with *Ubx* TREs in wing imaginal discs described in (C).

sense and antisense TRE transcripts. Heat treatment of second-instar larvae resulted in ectopic transcription of TRE transcripts in all imaginal discs of third-instar larvae. Ectopic transcription of each TRE transcript nucleated ectopic transcription of *Ubx* in wing imaginal discs (Fig. 6, C and D) (figs. S15 and S16) and facilitated the recruitment of Ash1 to the corresponding *Ubx* TREs (Fig. 6E). It is noteworthy that ectopic TRE transcription in second-instar larvae caused lethality in pupae. In contrast, ectopic *Ubx* expression was not observed in discs prepared from heat-treated parental strains and discs transcribing antisense TRE transcripts (Fig. 6C).

Transcription of antisense TRE transcripts attenuated endogenous transcription of *Ubx* in wing discs isolated from young third-instar larvae, which suggests that ectopic transcription of antisense RNA interferes with the TRE transcript–mediated recruitment of Ash1 to *Ubx* TREs. In summary, our data provide evidence that noncoding TRE transcripts facilitate activation of *Ubx* expression by recruiting Ash1 to the *Ubx* TREs in the fly.

Discussion. Noncoding RNAs play an important role in the recruitment of proteins in several epigenetic phenomena. Recent studies have linked siRNAs to heterochromatin formation and transcriptional silencing of transgenes and transposons (*29*, *30*). SiRNAs facilitate the recruitment of HMTs and DNA methyltransferases to chromatin (*31*, *32*). In *Schizosaccharomyces pombe*, heterochromatic silencing involves the RNA-induced initiator of transcriptional gene silencing complex (RITS), which contains an siRNA component that is essential for the recruitment of RITS to heterochromatic loci (*31*). The inability of RNase III, the key enzyme of the RNAi machinery, to degrade TRE transcripts into siRNAs and the interaction of Ash1 with full-length TRE transcripts in chromatin strongly argues against the involvement of siRNAs in the described RNA-dependent recruitment of Ash1 to chromatin.

Long ncRNAs are key players in imprinting and gene dosage compensation (*22*, *27*, *33*). In *Drosophila*, gene dosage compensation is achieved by a global twofold up-regulation of transcription from the male X chromosome and depends on the activity of the dosage compensation complex (DCC) that contains male-specific proteins and two ncRNAs, *RNA on X 1* (*rox1*) and *RNA on X 2* (*rox2*) (*20*). Both RNAs are transcribed by single-copy genes that, as well as several other X chromosome regions, serve as chromatin entry sites for the DCC on paternal X chromosomes (*20*, *27*). *Rox1* and *Rox2* facilitate the assembly and recruitment of the DCC to chromatin entry sites (*20*). In mammals, spreading of *Xist* RNA culminates in X chromosome inactivation (*22*). Current models propose that the association between ncRNAs and chromatin involves their interaction with proteins, nascent transcripts at template DNA, or the template DNA (*27*, *34*). The observed attenuation of the association between TRE transcripts and TREs by RNase H suggests that TRE transcripts are retained at TREs through hybridization with the corresponding template DNA. Because none of the known DNA repair systems targets DNA-RNA hybrids, RNA-DNA hybrids represent stable molecular entities that, in general, may anchor ncRNAs at corresponding DNA templates in chromatin (*35*).

The three TRE transcripts of *Ubx* do not share common sequence motifs. This is not surprising, because the functionally redundant *rox* RNAs and functionally identical regions in *Xist*, which are required for chromatin localization and protein recruitment, lack identifiable sequence motifs (*27*). Because many RNA-protein interactions are facilitated by RNA secondary structures, the interaction of Ash1 with TRE transcripts might be mediated by secondary RNA structures rather than sequence motifs. In addition, the specificity of RNA-protein interactions is often generated by induced-fit mechanisms that involve complex, extensive conformational changes in both proteins and the target RNA generating a specific interaction surface (*36*, *37*).

Rox1 and *rox2* RNAs transcribed from autosomes can localize to and mediate gene dosage compensation on the male X chromosome, indicating that the chromatin entry of *rox* RNAs does not depend on transcription of chromatin entry sites in cis (*38*). Thus, the association of transiently transcribed TRE transcripts with TREs in S2 cells suggests that TREs function as chromatin entry sites for the corresponding TRE transcripts in trans and cis, and that the transcription and

chromatin entry site activities of TREs are functionally separated. Cumulatively, our results support a model in which RNAs transcribed from the TREs of *Ubx* are retained at TREs through DNA-RNA interactions and provide a RNA scaffold that is bound by Ash1.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/311/5764/1118/DC1
Materials and Methods
Figs. S1 to S16
Tables S1 to S3
References

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A Swimming Mammaliaform from the Middle Jurassic and Ecomorphological Diversification of Early Mammals

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A docodontan mammaliaform from the Middle Jurassic of China possesses swimming and burrowing skeletal adaptations and some dental features for aquatic feeding. It is the most primitive taxon in the mammalian lineage known to have fur and has a broad, flattened, partly scaly tail analogous to that of modern beavers. We infer that docodontans were semiaquatic, convergent to the modern platypus and many Cenozoic placentals. This fossil demonstrates that some mammaliaforms, or proximal relatives to modern mammals, developed diverse locomotory and feeding adaptations and were ecomorphologically different from the majority of generalized small terrestrial Mesozoic mammalian insectivores.

The Middle Jurassic mammalian diversification gave rise to several emergent clades: basal eutriconodontans, amphitheriid cladotherians, the basal mammalian lineage of shuotheriids, and basal australosphenidans (*1*–*5*). These new clades of crown Mammalia coexisted with several mammaliaform lineages (the proximal relatives to modern mammals) (*1*, *6*–*8*). Docodontans are a Mesozoic mammaliaform lineage that have specialized molars for omnivorous feeding; several taxa are known from the Middle Jurassic (*1*, *9*–*13*). Here, we report on a large docodontan mammaliaform that has some dental features for feeding on aquatic invertebrates and small vertebrates, plus specialized skeletal and soft-tissue features for swimming and burrowing.

Description and comparison. *Castorocauda lutrasimilis*, gen. et sp. nov. (*14*), is from the Middle Jurassic Jiulongshan Formation, dated to be approximately 164 million years ago (*15*–*17*). The fauna includes pterosaurs (*17*, *18*), a coelurosaurian dinosaur (*19*), lissamphibians (*20*), abundant fossil insects (*21*), and the conchostracan *Euestheria* (*22*). The holotype of *C. lutrasimilis* (Fig. 1) is represented by a partial skeleton (preserved rostrum-tail length ≥425 mm) with incomplete cranium (preserved length ≥60 mm) but well-preserved mandibles and lower dentition (incisors 4, canine 1, premolars 5, molars 6). Lower molars 3 to 6 have the diagnostic characteristics of docodontans (Fig. 2): anteriorly placed and enlarged lingual cusp g, triangulated crests formed by cusps a-c and a-g, and two partially enclosed basins formed respectively by cusps a, b, and g, and by cusps a, c, and d (*9*–*12*). As in all docodontans, the molars were capable of both shearing by the triangulated crests and grinding between the anterior (''pseudotalonid'') basin and the transversely widened upper molars (*9*–*12*). *Castorocauda* is distinctive from other docodontans in having mediolaterally compressed crowns of molars 1 and 2, each with five cusps in straight alignment (*23*, *24*); primary cusp a and posterior cusps c and d are slightly recurved (Fig. 2). These ''triconodont-like'' anterior molars are plesiomorphic for mammaliaforms (*6*–*8*) but nonetheless distinctive among docodontans. They are convergent to those of placental mesonychians and Eocene whales (*25*). This type of molar with recurved cusps in alignment is hypothesized to be a specialization for feeding on fish and aquatic invertebrates by functional analogy to the teeth of modern pinniped carnivores such as seals.

Castorocauda is preserved with intact middle ear bones (Fig. 2) on the mandible, including the articular (malleus), the surangular, and the angular (ectotympanic). The middle ear bones in anatomical association with the mandible corroborate a previous interpretation of the middle ear in docodontans (*26*). A concavity on the

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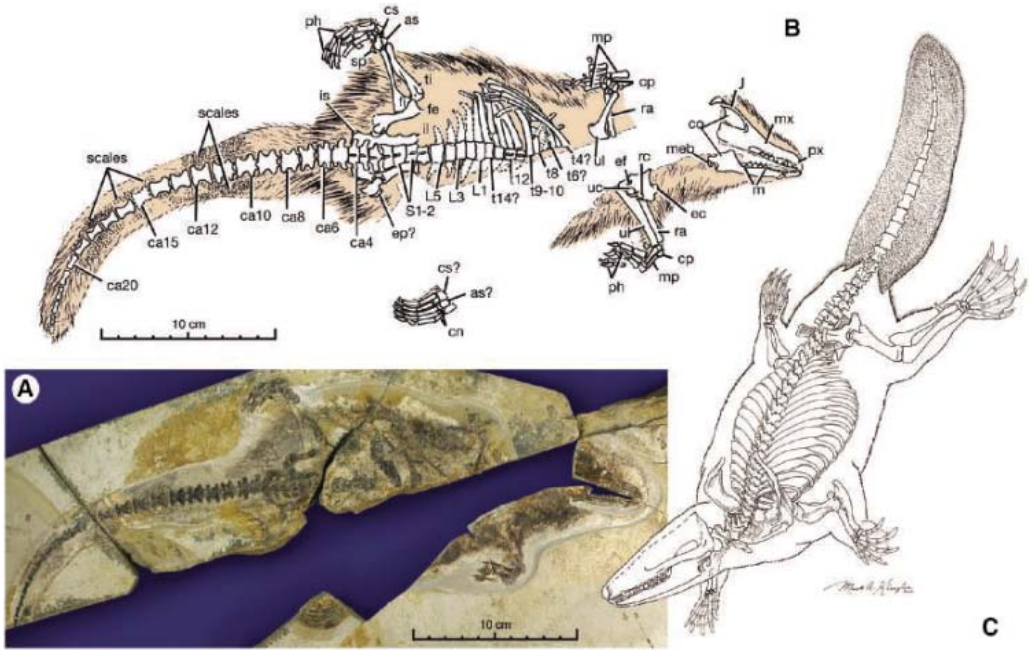

Fig. 1. Holotype of *Castorocauda lutrasimilis* [Jinzhou Museum of Paleontology (JZMP) 04-117]. (**A**) Photograph of the holotype. (**B**) Osteological structures and preserved soft-tissue features. (**C**) Reconstruction of *Castorocauda lutrasimilis* as a swimming and burrowing mammaliaform. Abbreviations: as, astragalus; ca, caudal vertebrae; cn, ento-, meso-, and ecto-cuneiforms; co, coronoid process of dentary; cp, carpals; cs, calcaneus; ec, ectepicondyle and supinator shelf (humerus); ef, entepicondyle foramen; ep?, probable epipubis; is, ischium; j, jugal; L1-6, lumbar ribs 1 to 6; m, molars; mb, manubrium of malleus; mp, metacarpals; mx, maxilla; px, premaxilla; ra, radius; rc, radial condyle; S1-2, sacrals 1 and 2; sp, extratarsal ("poisonous") spur; t4-t14 (preserved ribs through thoracic 17); uc, ulnar condyle; ul, ulna.

posterior aspect of the mandibular angle accommodates the ectotympanic (angular). The posterior position of the ectotympanic concavity on the mandibular angle in docodontans is different from and more derived than that in the mammaliaforms *Sinoconodon* and *Morganucodon*, in which the ectotympanic concavity is on the medial aspect of the mandibular angle (*6*, *7*). The manubrium of the malleus (retroarticular process of the articular) is anteriorly curved and long in comparison with the short manubrium of *Morganucodon* and *Sinoconodon* (*6*, *7*, *27*). The proportion of the malleus manubrium is similar to that of extant monotremes, although slightly more robust than in the latter. *Castorocauda* is similar to crown Mammalia and more derived than *Sinoconodon*, *Morganucodon*, and all pre-mammaliaform cynodonts (*27*) in preserved middle ear features.

Our analyses, including new characters of *Castorocauda*, corroborate that docodontans are a mammaliaform clade, less derived than *Hadrocodium* but more derived than *Sinoconodon* and *Morganucodon* (*1*, *8*, *26*, *28*, *29*). Among docodontans, *Castorocauda* is closely related to the Middle Jurassic *Krusatodon* and *Simpsonodon* of England (*9*–*11*), suggesting interchange between faunas of the Eurasian landmasses during the Middle Jurassic time.

Integument. The fur of *Castorocauda* is preserved as impressions of guard hairs and carbonized under-furs. Hairs and hair-related integument structures are important characteristics of all modern mammals (*30*, *31*). Several younger fossils within the crown Mammalia are preserved with fur, including basal eutherians and metatherians (*32*, *33*), multituberculates, eutriconodontans, and symmetrodonts (*1*). This indicates that the presence of fur is ancestral for the crown Mammalia. *Castorocauda* further shows that fur was also present in mammaliaform relatives of modern mammals (Fig. 3A) and that the origins of biological adaptations of mammalian integument, such as tactile sensory function and thermal insulation, occurred before the origin of the crown Mammalia (*30*, *31*).

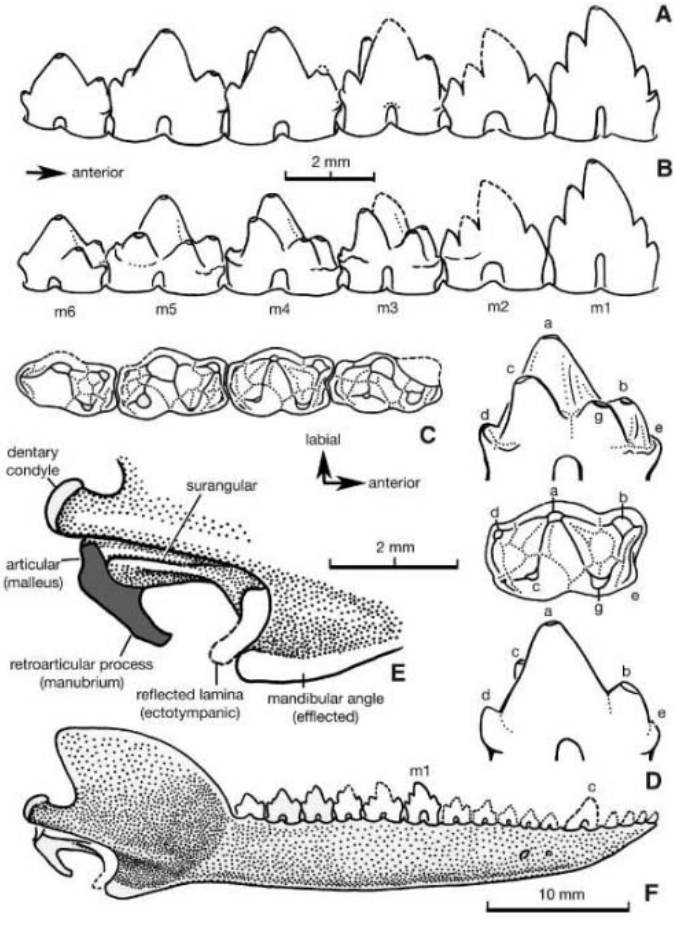

Fig. 2. Dentition and mandible of *Castorocauda lutrasimilis* (JZMP04-117). (**A**) Labial view of lower molars 1 to 6. (**B**) Lingual view of lower molars 1 to 6. (**C**) Crown view of lower molars 3 to 6. (**D**) Cusp pattern [cusp designation from (*11*, *12*)]. (**E**) Middle ear bones. (**F**) Reconstructed mandible and middle ear bones (lateral view). c, canine; m, molars.

Swimming and fossorial adaptations. Mammals with fossilized pelage from the Early Cretaceous Yixian Formation have few or no hairs on the tail posterior to the pelvic area, indicating that their tails were naked or scaly. By contrast, *Castorocauda* shows a broad outline of preserved fur on the tail, which is at least 50% wider than the pelvic width along the length of the tail. Carbonized scales are present adjacent to caudal vertebrae 9 through 20 but are best seen on both sides of caudals 11 through 18 (Fig. 1). The proximal 25% of the tail is covered by guard hairs, the middle 50% mostly covered by scales with sparse hairs, and the distal 25% by scales interspersed with guard hairs. The broad and scaly tail of *Castorocauda* was similar to that of the modern beaver *Castor canadensis*, a semiaquatic placental mammal well adapted for swimming.

Postcranial skeletal features of *Castorocauda* also show specializations for swimming. Caudal vertebrae 5 through 13 have dorsoventrally compressed centra, with the more posterior vertebrae completely flattened. Caudal vertebrae 5 through 15 have bifurcate transverse processes. Both are key features of placental mammals with tails specialized for swimming (*34*). In caudal vertebrae 5 through 7, the cranial transverse process is much longer than the caudal transverse process. The flattened centrum and bifurcate transverse processes form a distinctive "butterfly" pattern (Fig. 4). This is identical to caudals 9 through 12 of the beaver (Fig. 4). In caudals 7 through 10, the cranial and caudal transverse processes are of approximately equal size and are similar to caudals of the river otter *Lutra canadensis*. In caudals 10 through 18, the transverse processes are reduced and the vertebral outline is graded into an hourglass shape. These vertebral and tail characteristics in this Middle Jurassic docodontan are very similar to those of modern beavers and otters, mammals capable of paddling and/or dorsoventral caudal undulation for propulsion in swimming (*34*, *35*). Remnants of soft tissue between pedal digits suggest some webbing of hind feet.

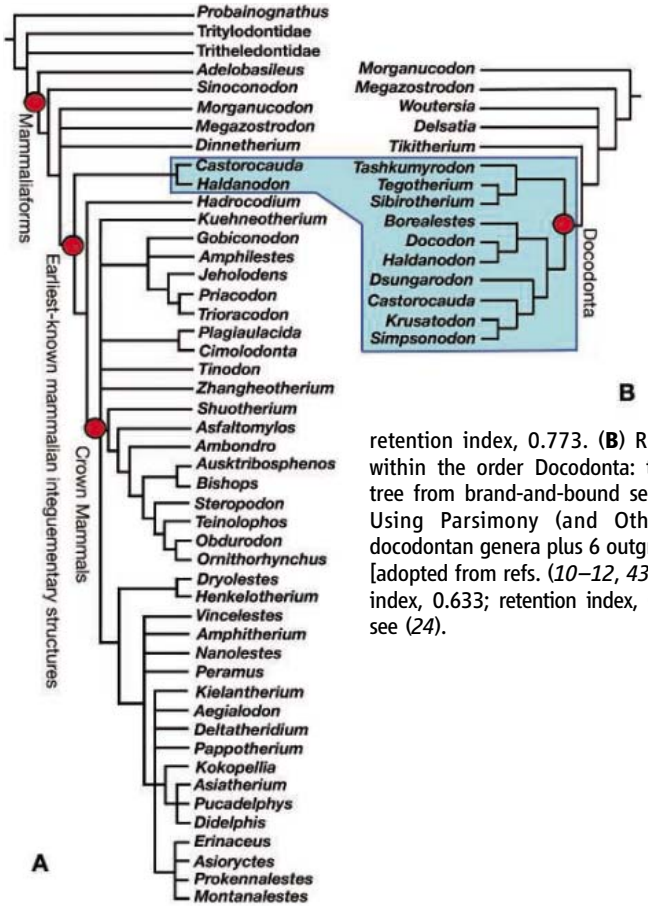

Fig. 3. Phylogenetic relations of the docodontan *Castorocauda lutrasimilis*. (**A**) Relation of *Castorocauda* to other mammaliaforms (including crown Mammalia): the strict consensus of 74 equally parsimonious trees from 1000 heuristic runs of PAUP (version 4.0b10) search of a data set of 48 taxa and 281 characters [emended from refs (*1*, *28*, *29*, *42*, *43*)]; for each of the 78 parsimonious trees: tree length, 941; consistency index, 0.507; retention index, 0.773. (**B**) Relationships of *Castorocauda* within the order Docodonta: the single most parsimonious tree from brand-and-bound search of Phylogenetic Analyses Using Parsimony (and Other Methods) 4.0b10 of 9 docodontan genera plus 6 outgroups and 19 dental characters [adopted from refs. (*10*–*12*, *43*)]; tree length, 49; consistency index, 0.633; retention index, 0.755. For details of analyses, see (*24*).

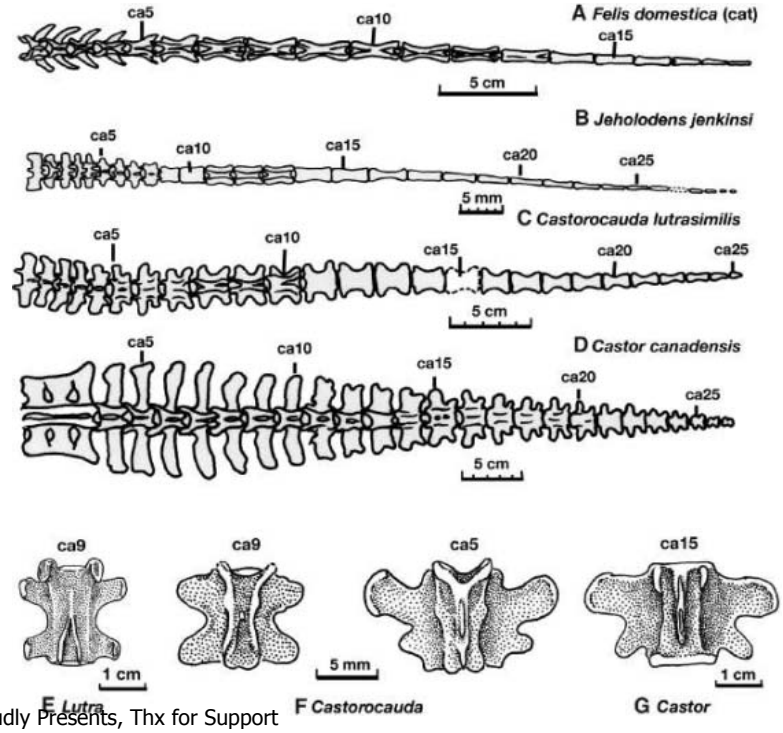

Fig. 4. Comparison of the caudal vertebrae of *Castorocauda lutrasimilis* and other mammals (A to E scaled to about the same tail length, all dorsal view). (**A**) *Felis domestica* (cat), a generalized terrestrial mammal. (**B**) *Jeholodens jenkinsi* (eutriconodontan), a terrestrial mammal of the Early Cretaceous. (**C**) *Castorocauda* (JZMP04117), all caudal vertebrae. (**D**) *Castor canadensis* (modern beaver), all caudal vertebrae. (**E**) *Lutra canadensis* (river otter), caudal 9. (**F**) *Castorocauda*, caudals 5 and 9. (**G**) Beaver, caudal 15. Transverse processes are present in proximal caudal vertebrae of all mammals but are reduced markedly in the vertebrae posterior to the pelvis and are absent in more distal caudal vertebrae of generalized terrestrial mammals (e.g., *Felis* and *Jeholodens*). In semiaquatic placentals, the hypertrophied and bifurcate transverse processes form a "butterfly" or "horizontal H" pattern in caudal vertebrae that are correlated with the scaly and paddlelike tail of the beaver (*34*). The Middle Jurassic *Castorocauda* developed similar caudal vertebral structures and had a broad, flattened, scaly tail as in modern beavers. Its semiaquatic feeding adaptation is similar to that of the modern river otter.

Both *Castorocauda* and the Late Jurassic docodontan *Haldanodon* are similar to the modern monotreme *Ornithorhynchus* in forelimb fossorial specializations (*28*–*36*). The distal humerus is wide; it has hypertrophied epicondyles, a supinator process, and massive and widely separated ulnar and radial condyles. The ulna has a massive and asymmetrical olecranon process. The radius is robust. The carpals are blocklike, and the metacarpals and proximal phalanges are robust and wide. A single and large sesamoid bone for the digital flexor muscle tendon is present at the metacarpal-phalangeal joint.

The forelimb of *Ornithorhynchus* is adapted to digging and also used for rowing during swimming and diving (*35*–*37*). It has been hypothesized that the docodontan *Haldanodon* was semiaquatic (*28*, *38*). From the additional evidence of *Castorocauda*, it appears that many docodontans were burrowing mammals with sprawling limb posture and gait in terrestrial locomotion. They may have also used the forelimbs for rowing during swimming, as an exaptation (*37*), as in the platypus.

Plated ribs. Based on the preserved ribs and vertebral bodies, we estimate that *Castorocauda* probably had 14 thoracic, 7 lumbar, 3 sacral and 25 caudal vertebrae (Fig. 1). The proximal portions of the thoracolumbar ribs have broad costal plates; the adjacent costal plates overlap by at least one-third of the plate width. The costal plates resemble the plated thoracolumbar ribs of the cynodonts *Thrinaxodon*, *Cynognathus* (*39*), and *Diademodon* (*40*), although *Castorocauda* differs in lacking the costal tubercles (ridges) and in the absence of interlocking of adjacent costal plates seen in *Cynognathus* and *Diademodon* (*39*). Among Mesozoic mammals, the gobiconodontid *Repenomamus* has costal plates in the anterior lumbar and posterior thoracic ribs, but these are far less developed than those in *Castorocauda*.

Plated ribs have a homoplastic distribution among cynodonts and mammaliaforms. They are absent in the traversodontids but present in the closely related diademodontids. Costal plates are absent in many intermediate groups between primitive cynodonts *Diademodon*, *Cynognathus*, and *Thrinaxodon*, and the more derived *Castorocauda*. It is parsimonious to hypothesize that *Castorocauda* represents a reversal (or convergence) to cynodonts in the development of costal plates. Plated lumbar ribs probably increase the insertion area for the M. iliocostalis muscle and reinforce the support of the adjacent vertebral segments by interlocking adjacent ribs, thereby strengthening the trunk (*39*). Thoracolumbar rib plates are present (although much narrower) in xenarthran mammals with either fossorial or arboreal adaptations (*39*). The plated ribs of *Castorocauda* are very thin and lack the pachyosteosclerosis (hypertrophied growth of the highly compact bones for buoyancy control), a characteristic of fully aquatic and much larger sirenian mammals.

Castorocauda is the largest known Jurassic mammaliaform (including mammals). By its preserved skull length of ≥60 mm and the well-established scaling relation of skull and body mass (*8*, *41*), we estimate that the body mass of the holotype specimen was at least 500 g. The preserved length from rostrum to tail is 425 mm, but the actual body length is certainly greater. The length of female platypuses with similar fossorial and semiaquatic habits ranges from 390 to 550 mm, corresponding to a body mass range of 700 to 2400 g. We estimate the upper limit of body mass to be approximately 800 g for *Castorocauda*. All other Jurassic mammals are small (*1*). Constrained by their small size, most were generalized terrestrial insectivores or omnivores. Previously, the largest taxon was *Sinoconodon rigneyi* (*7*); its largest individuals reached an estimated body mass of 500 g (*8*). Based on its relatively large size, swimming body structure, and anterior molars specialized for piscivorous feeding, *Castorocauda* was a semiaquatic carnivore, similar to the modern river otter. This fossil shows that basal mammals occupied more diverse niches than just those of small insectivorous or omnivorous mammals with generalized terrestrial locomotory features. *Castorocauda* also suggests that mammaliaforms developed physiological adaptations associated with pelage, well before the rise of modern Mammalia, and had more diverse ecomorphological adaptations than previously thought, with at least some lineages occupying semiaquatic niches.

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14. Etymology: *Castor* (Latin), beaver; *cauda* (Latin), tail; after the broad, flattened, scaly, and beaverlike tail for swimming; *lutra* (Latin), otter; *similis* (Latin), similar; similar to extant otters in some dental and vertebral characters. Systematics: Clade Mammaliaformes (Class Mammalia by traditional definition); Order Docodonta; Family *incertae sedis*; gen. et sp. nov. *Castorocauda lutrasimilis*. Holotype: Jinzhou Museum of Paleontology, Jinzhou City, Liaoning Province, China (JZMP-04-117), an incomplete, flattened skeleton, partial skull, preserved with fur and scales. Locality and Age: Daohugou locality (N41°18.979′, E119°14.318′), Ningcheng County, Inner Mongolia, China; Jiulongshan Formation, dated to be 164 million years ago (*15*–*17*).
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23. Diagnosis: Lower dentition i4, c1, p5, m6 (Fig. 2). *Castorocauda lutrasimilis* has the typical docodontan molar features in molars 3 to 6 but is distinguishable from other docodontans by the mediolaterally compressed molars 1 and 2 with slightly recurved cusps in alignment. In mandibular length, *C. lutrasimilis* is about twice as large as *Docodon*, the next largest docodontan. See also (*24*).
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References

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X-ray Flares from Postmerger Millisecond Pulsars

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Recent observations support the suggestion that short-duration gamma-ray bursts are produced by compact star mergers. The x-ray flares discovered in two short gamma-ray bursts last much longer than the previously proposed postmerger energy-release time scales. Here, we show that they can be produced by differentially rotating, millisecond pulsars after the mergers of binary neutron stars. The differential rotation leads to windup of interior poloidal magnetic fields and the resulting toroidal fields are strong enough to float up and break through the stellar surface. Magnetic reconnection–driven explosive events then occur, leading to multiple x-ray flares minutes after the original gamma-ray burst.

Gamma-ray bursts (GRBs) are flashes of gamma rays occurring at the cosmological distances. They fall into two classes (*1*): short-duration (<2 s) hard-spectrum bursts and long-duration soft-spectrum bursts. Long GRBs result from core collapses of massive stars (*2*), and short GRBs appear to be produced in mergers of neutron star binaries or black hole–neutron star binaries (*3*–*9*). Recently, thanks to accurate localizations of several short GRBs (*3*, *6*, *8*) by satellites Swift and High Energy Transient Explorer 2 (HETE-2), the multiwavelength afterglows from these events have been detected and the associated host galaxies have been identified. The observations provide a few pieces of evidence in favor of the binary compact object merger origin of short GRBs (*10*–*12*). Because it takes ~0.1 to 1 billion years of gravitational wave radiation before the binary coalesces, at least some short GRB host galaxies should contain a relatively old stellar population. Because neutron stars in the binary system usually receive a very high natal velocity, the merger site is preferably at the outskirt of the host galaxy, and the circumburst medium density is likely low. These characteristics have been revealed by recent observations: First, the identified elliptical galaxies associated with GRB 050509B (*3*, *4*) and GRB 050724 (*8*, *9*) suggest that these hosts are early-type galaxies with a low star-formation rate, ruling out progenitor models invoking active star formation. Second, the nondetection of any supernova signal from GRB 050709 indicates that short bursts are not associated with collapses of massive stars (*5*, *7*). Third, afterglow modeling of GRB 050709 suggests a low-density environment (*13*), which is consistent with that of the outskirt of the host galaxy or that of an intergalactic medium.

However, the above merger origin was recently challenged by the discovery of x-ray flares occurring after two short bursts. X-ray flares were discovered to occur at least ~100 s after the triggers of the short GRB 050709 (*5*) and GRB 050724 (*8*). These flares require that the central engine is in long-lasting activity. This requirement conflicts with the current models involving neutron star–neutron star mergers (*14*, *15*) or neutron star–black hole mergers (*16*), because all of these models are attached to a common postmerger picture that invokes a black hole surrounded by a torus. The predicted typical time scales for energy release are much shorter than ≥100 s, as observed in GRBs 050709 and 050724. Therefore, understanding the origin of x-ray flares from short bursts is currently of great interest. Here, we show that such flares can be produced by differentially rotating, millisecond pulsars with typical surface magnetic fields that occur after the mergers of binary neutron stars.

In the conventional scenarios of short bursts (*10*–*12*), after the merger of a neutron star binary, a stellar-mass black hole is formed with a transient torus of mass ~1 to 10% of the total. These scenarios are valid if the total mass (~2.5 to 2.8 $M_\odot$, where $M_\odot$ is the solar mass) of the postmerger object is larger than the maximum mass of a nonrotating Tolman-Oppenheimer-Volkoff neutron star, $M_{\rm max,0}$. This is valid if the nuclear equation of state (EOS) is soft to moderately stiff (*17*). However, the total mass of the postmerger object is smaller than $M_{\rm max,0}$ for very stiff EOSs on the basis of mean field theory (*17*). Timing observations of the millisecond pulsar J0751+1807 in a circular binary system with a helium white-dwarf companion (*18*) reveal the existence of a neutron star with mass of $2.1 \pm 0.2\ M_\odot$ (at the 1σ confidence level). This measurement implies that the maximum mass of nonrotating neutron stars must be larger than $2.1\ M_\odot$ so that stiff EOSs are favored. Furthermore, recent general relativistic numerical simulations (*17*, *19*) have shown that for stiff to very stiff nuclear EOSs, the postmerger object is indeed a differentially rotating massive neutron star with period of ~1 ms, because uniform rotation and differential rotation can support a maximum mass ~20 and ~50% higher than $M_{\rm max,0}$, respectively. It is therefore reasonable to assume the existence of a differentially rotating millisecond pulsar after a double neutron star merger. Such a pulsar should also be surrounded by a hot torus with mass ~0.01 to 0.1 $M_\odot$. Similar to the previous scenarios, a short burst may be produced by the Parker instability in the torus (*11*) or the annihilation of neutrinos emitted from the torus (*12*).

After the GRB trigger, differential rotation starts to wind the interior magnetic field into a toroidal field (*20*, *21*). To represent physical processes of windup and floating of the magnetic field, we considered a simple two-component model in which the star is divided into two zones with a boundary at the radius $R_c \cong 0.5\ R_*$ (where R_* is the stellar radius): the core and the shell components. Their moments of inertia are I_c and I_s and their angular (rotation) velocities are Ω_c and Ω_s, respectively. The differential angular velocity is then $\Delta\Omega = \Omega_c - \Omega_s$ and its initial value (marked by a subscript zero) is taken as $(\Delta\Omega)_0 = A_0\Omega_{s,0}$ (where A_0 is the ratio of the initial differential angular velocity to the shell's initial angular velocity). If the radial magnetic field compo-

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nent is B_r, then the toroidal field component B_ϕ increases as

$$\frac{dB_\phi}{dt} = (\Delta\Omega)B_r \quad (1)$$

There is a magnetic torque, $T_m = (2/3)R_c^3 B_r B_\phi$, acting between the core and shell (*22*). This torque opposes the differential rotation. Another torque results from magnetic dipole radiation, $T_d = 2B_s^2 R_*^6 \Omega_s^3/(3c^3)$, where c is the speed of light and $B_s = \varepsilon B_r$ (here, ε is defined by the ratio of the effective surface dipole field strength to the radial field strength). Under action of these two torques, the angular velocities of the shell and the core components evolve according to

$$I_s \frac{d\Omega_s}{dt} = T_m - T_d \quad (2)$$

and

$$I_c \frac{d\Omega_c}{dt} = -T_m \quad (3)$$

respectively. The torque from magnetic dipole radiation can be neglected if $B_\phi \gg B_r \varepsilon^2 (R_*/R_c)^3 (R_*\Omega_s/c)^3$. This condition is easily satisfied at the time $t \gg t_0 \equiv \varepsilon^2 A_0^{-1} \Omega_{s,0}^{-1} (R_*/R_c)^3$ (where t_0 is $\sim$ 0.2 ms for typical parameters). Thus, from Eqs. 1 to 3, we obtained

$$\frac{d^2\Delta\Omega}{dt^2} = -\frac{2I}{3I_c I_s} R_c^3 B_r^2 (\Delta\Omega) \quad (4)$$

where $I = I_c + I_s$ is the total moment of inertia of the star. Letting

$$\tau = \left(\frac{2I}{3I_c I_s} R_c^3 B_r^2\right)^{-1/2} \simeq 2.3 \times 10^5 (\varepsilon/0.3) B_{s,8}^{-1} \text{ s} \quad (5)$$

where $I_c \cong I_s = 10^{45}$ g · cm^2 and $R_* = 10^6$ cm are taken and $B_{s,8}$ is in units of 10^8 G, we found a solution of Eq. 4:

$$\Delta\Omega = A_0 \Omega_{s,0} \cos(t/\tau) \quad (6)$$

This indicates that differential rotation would behave as a resonator if there were no energy dissipation.

The increasing toroidal field becomes unstable because of the buoyancy effect when $B_\phi = B_b \cong 10^{17}$ G (*20*). This corresponds to the time

$$t_b = \frac{B_b}{B_r A_0 \Omega_{s,0}} \simeq 4.8 \times 10^4 (\varepsilon/0.3) \times B_{s,8}^{-1} A_0^{-1} P_{s,0,ms} \text{ s} \quad (7)$$

where $P_{s,0,ms}$ is the initial spin period of the shell component in units of milliseconds. Comparing Eqs. 5 and 7, we see that t_b is substantially less than τ for $A_0^{-1} P_{s,0,ms} \leq 1$ and that the differential angular velocity $\Delta\Omega$ is approximately constant until the time t_b. At this time, the buoyant force is just equal to the force from antibuoyant stratification existing in the star. As the time increases, the buoyant force acting on the toroid would begin to exceed the antibuoyant force, and the toroid will float up toward the stellar surface. The net force density acting on this toroid is given by

$$f_b = \frac{B_r B_b A_0 \Omega_{s,0} (t - t_b)}{4\pi c_s^2} g \quad (8)$$

where c_s is the speed of sound of the embedding medium and g is the surface gravity. In terms of Eq. 8 and Newton's second law, we obtained the buoyancy time scale for the toroid to float up and penetrate through the stellar surface:

$$\Delta t_b = \left[\frac{12\pi\rho R_* c_s^2}{B_r B_b g A_0 \Omega_{s,0}}\right]^{1/3} \simeq 0.26(\varepsilon/0.3)^{1/3} \times B_{s,8}^{-1/3} A_0^{-1/3} P_{s,0,ms}^{1/3} \text{ s} \quad (9)$$

where $\rho \cong 10^{14}$ g cm^{-3} is the mass density of the embedding medium, and the typical values of the speed of sound and the surface gravity are 10^{10} cm s^{-1} and 10^{14} cm s^{-2}, respectively. This time scale is much shorter than t_b, suggesting that the toroid, after its field strength reaches B_b, would rapidly float up to the stellar surface.

Once penetrating through the surface, the toroidal fields with different polarity may reconnect (*20*), giving rise to an explosive event. Its energy is

$$E_b = \frac{B_b^2}{8\pi} V_b \simeq 1.6 \times 10^{51} \text{ergs} \left(\frac{V_b}{V_*}\right) \quad (10)$$

where V_b and V_* are the toroid's volume and the stellar volume, respectively. This energy depends on the toroid's volume rather than on the initial magnetic field and the stellar spin period. An upper limit to the outflow mass ejected is estimated by

$$M_{b,max} = f_b V_b / g \simeq 0.9 \times 10^{-7} M_\odot (\varepsilon/0.3)^{-2/3} \times B_{s,8}^{2/3} A_0^{2/3} P_{s,0,ms}^{-2/3} \left(\frac{V_b}{V_*}\right) \quad (11)$$

Because of an initial huge optical depth, the outflow will expand relativistically and its minimum average Lorentz factor is

$$\Gamma_{b,min} \simeq 1.0 \times 10^4 (\varepsilon/0.3)^{2/3} B_{s,8}^{-2/3} A_0^{-2/3} P_{s,0,ms}^{2/3} \quad (12)$$

The x-ray flares observed at $t_{flare} \sim t_b \cong 100$ s after GRBs 050709 and 050724 require that the surface magnetic field of a central pulsar $B_s \sim 4.8 \times 10^{10} (\varepsilon/0.3) A_0^{-1} P_{s,0,ms} (t_{flare}/100 \text{ s})^{-1}$ G. For typical values (*19*, *22*) of the model parameters (i.e., $A_0 \sim 1$, $P_{s,0} \sim 1$ ms, and $\varepsilon \sim 0.3$), this field strength is in the range of the surface magnetic fields of isolated pulsars. Furthermore, it is characteristic of the stellar magnetic field that has decayed in ~0.1 to 1 billion years before the merger of a neutron star binary (*23*). Inserting this field into Eq. 12, we found the minimum average Lorentz factor of the outflow from a magnetic reconnection–driven explosion, $\Gamma_{b,min} \sim 160(t_{flare}/100 \text{ s})^{2/3}$, showing that the outflow is ultrarelativistic. After the end of this event, a similar windup of the interior magnetic field with B_r would start again following the same processes described above, leading to another explosion.

Collisions among the outflows with different Lorentz factors would produce late internal shocks and x-ray flares (*24*, *25*). These shocks must produce lower energy photons than did the earlier internal shocks during the prompt GRB phase. For the internal shock model, the characteristic synchrotron frequency is $\nu_m \propto L^{1/2} R_{sh}^{-1} \propto L^{1/2} \Gamma_b^{-2} \delta t^{-1}$ (where L is the luminosity, R_{sh} is the shock radius, Γ_b is the bulk Lorentz factor, and δt is the time interval between two adjacent energy shells that the central engine ejects). The late, soft flare is the result of the combination of a lower luminosity and a longer time interval (than that of the prompt emission, where $\delta t \sim t_b$ in our flare model). In addition, as the stellar differential rotation weakens (i.e., A_0 decreases), the time interval δt and the outflow's Lorentz factor Γ_b increase (see Eqs. 7 and 12). Because the maximum flux density of the synchrotron radiation scales as $F_{\nu,max} \propto \Gamma_b^{-3}$ (*24*), the flux density at frequency ν is $F_\nu = F_{\nu,max} (\nu/\nu_m)^{-(p-1)/2} \propto \Gamma_b^{-(2+p)} \delta t^{-(p-1)/2}$ for $\nu > \nu_m$ in the slow-cooling case (where p is the spectral index of the shock-accelerated electrons) (*26*). Thus, the flare occurring at later times has a smaller flux density because of the larger Lorentz factor and longer time interval. This result is consistent with the observed reduced flaring activity of GRB 050724. Therefore, our model can provide a self-consistent explanation for all the observations including the energetics (see Eq. 10) and the temporal and spectral properties of the x-ray flares.

Generally speaking, the surface magnetic field of the postmerger pulsar could have a wider range than the preferred value invoked here to interpret the ~100-s flares in GRBs 050709 and 050724. For stronger fields, this would give rise to multipeaks in the prompt phase (as observed in some short GRBs) or, if the flares are not bright enough, they may be masked by the steep decay component of the prompt emission tail (*25*). For weaker fields, the putative flares occur much later and are energetically insignificant. This would give rise to smoother x-ray afterglow lightcurves as in several GRBs observed by Swift (e.g., GRB 050509B) (*3*).

X-ray flares were observed in nearly half of long Swift bursts (*27*, *28*). Even though the two classes of bursts have different progenitors (namely collapsars for long bursts and binary neutron star mergers for short bursts), similar temporal properties (e.g., peak times and temporal indices before and after the peaks) suggest that the x-ray flares may have a common origin. Therefore, we suggest that some long bursts may originate from moderately magnetized millisecond pulsars with hyperaccreting accretion disks after the collapses of massive stars, and their x-ray flares are the result of strong interior differential rotation of these pulsars. The differences in duration, energetics, and spectrum for the two classes of bursts would be due to different accretion disks [e.g., a transient torus for short bursts (*10*–*12*) and a fall-back accretion disk for long bursts (*29*, *30*)]. When the surface magnetic fields are strong enough, the spin down of this central engine pulsar would provide energy injection to the postburst relativistic outflow (*31*), which could interpret the late x-ray humps detected in many GRBs (*25*, *28*).

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Explaining the Color Distributions of Globular Cluster Systems in Elliptical Galaxies

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The colors of globular clusters in most large elliptical galaxies are bimodal. This is generally taken as evidence for the presence of two cluster subpopulations that have different geneses. However, here we find that, because of the nonlinear nature of the metallicity-to-color transformation, a coeval group of old clusters with a unimodal metallicity spread can exhibit color bimodality. The models of cluster colors indicate that horizontal-branch stars are the main drivers behind the empirical nonlinearity. We show that the scenario gives simple and cohesive explanations for all the key observations and could simplify theories of elliptical galaxy formation.

One of the most outstanding discoveries from observations of elliptical galaxies over the past decade is the bimodal color distribution of globular clusters, gravitationally bound collections of millions of stars (*1*–*8*). The phenomenon is widely interpreted as evidence of two cluster subsystems with distinct geneses within individual galaxies (*9*). However, given the many ways of forming clusters in elliptical galaxies, it is surprising that the cluster color distributions behave in an orderly way. For instance, the numbers of blue and red clusters in large galaxies are roughly comparable (*1*–*8*); blue and red clusters are old (>10 billion years) and coeval (*9*) and differ systematically in spatial distribution and kinematics (*5*, *10*–*16*); and their relative fractions and peak colors strongly correlate with host galaxy properties (*2*–*8*). Here, we propose a simpler solution that does not necessarily invoke distinct cluster subsystems and has a sound basis in both empirical and theoretical relations between metallicity and colors.

A recent observation (*8*) reveals that the $g - z$ color (*17*) of clusters correlates with their [Fe/H] (*18*) (Fig. 1A). The observed relation is tight enough to show a notable departure from linearity with a slope rapidly changing at [Fe/H] ≈ -1.0. A closer inspection suggests that it might follow an inverted S–shaped "wavy" curve with a quasi-inflection point at [Fe/H] ≈ -0.8. To examine this indication, we overplotted predicted colors of 13-billion-years (Gy) clusters from two different models (*19*, *20*). Although there is good agreement between the models, they predict systematically redder colors for the [Fe/H] ≈ -1.0 clusters that serve as key part of the possible inverted-S shape of the observed relation. We present our equivalent 13-Gy models with the [Fe/H] grid spacing of Δ[Fe/H] = 0.1 (Fig. 1B), a resolution sufficient to sample the region of [Fe/H] ≈ -1.0. The main asset of our model is the consideration of the systematic variation in the mean color of horizontal-branch (HB) stars as a function of [Fe/H] (*21*–*24*). The version of our model that excludes the prescription for the systematic HB variation, although showing agreement with the other models, does not match the observations as well. However, by including the realistic HB variation we can reproduce the clusters with [Fe/H] ≈ -1.0 and, in turn, the observed wavy feature. This suggests that the wavy feature along the sequence defined by the observed clusters is real.

The observed wavy feature in the metallicity-color relation is a result of two complementary effects (Fig. 1B): (i) The integrated color of the stars before the HB stage (i.e., the main se-

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quence and the red giant branch stages) is a nonlinear function of metallicity at given ages, showing a mild departure from linearity at lower metallicity. (ii) The color of the HB changes at a brisk pace between [Fe/H] values about equal to −0.6 and −0.9, further strengthening the departure from linearity. As a result, the color becomes several times more sensitive to metallicity between [Fe/H] values of −0.6 and −0.9, resulting in a quasi-inflection point at [Fe/H] ≈ −0.8. The former effect is visible in all models, but the latter effect is present only in our model (Fig. 1, A and B). We illustrated the well-known effect of metallicity on the systematic HB color variation (Fig. 1C). The color of HB varies abruptly between [Fe/H] values of −0.6 and −0.9, where the HB just departs from the red-clump position. The physics of this phenomenon is described in (*24*). The Galactic globular clusters with HB morphology similar to the synthetic models include NGC 6624, NGC 104, NGC 6638, and NGC 5904 for [Fe/H] values of –0.4, –0.7, –1.0, and –1.3, respectively (*21*). We note that, because the range of the rapid change is as small as ~0.3 in [Fe/H], the wavy feature would not be present in the models with a [Fe/H] grid spacing larger than 0.3.

This nonlinear nature of the empirical relation between intrinsic metallicity and its proxy, colors, may hold the key to understanding the color bimodality phenomenon: The wavy feature brings about the bimodality by projecting equidistant metallicity intervals near the quasi-inflection point onto larger color intervals. To scrutinize this "projection effect," we have performed a Monte Carlo simulation using 100,000 coeval clusters (Fig. 2). For [Fe/H] distributions, we make a simple assumption of a broad Gaussian with standard deviation $\sigma_{[Fe/H]} = 0.5$ dex (Fig. 2, A and E). For a direct comparison with typical galaxies NGC 4649 and M87, we adopt the mean [Fe/H] of their entire globular clusters, $\langle[Fe/H]\rangle_{GC}$ (roughly −0.65), as inferred from the observed mean $g - z$ color (roughly 1.3) (*8*). Next, the $g - z$ color of each cluster is obtained by using our theoretical [Fe/H]-$g-z$ relation for the 13-Gy population. In the color-magnitude diagrams of 1000 randomly selected clusters (Fig. 2, B and F), two vertical bands of clusters are immediately visible at $g - z$ values roughly equal to 0.9 and 1.4. The resultant color histograms of 100,000 clusters (Fig. 2, C and G) show prominent dips near their centers, reproducing the observed histograms of NGC 4649 and M87 (Fig. 2, D and H). For comparison, the color distribution (Fig. 2G) that is obtained by using a simple linear (straight line) fit to the [Fe/H] versus $g - z$ data is in conflict with the observation. The quasi-inflection points are also apparent in other combinations of bandpasses, such as $V - I$ (Fig. 2I). As a result, with an identical [Fe/H] distribution to that in Fig. 2E, the model (Fig. 2J) reproduces the observed $V - I$ histogram (*5*) (Fig. 2L). These results are in good agreement with detailed studies indicating that the mean ages of both blue and red clusters in individual galaxies are old (>10 Gy) and comparable within a couple of Gy (*9*). Moreover, the comparable numbers of blue and red clusters found in large galaxies are due to the fact that the dip-colors of the histograms correspond to the midpoint of the color spanned by clusters in large galaxies.

Although photometry is used commonly to infer the metallicities of clusters, it is no substitute for spectroscopy. We wonder whether the observed distributions of metal indices, such as the *Mg b* absorption line (near 5170 Å), can also be explained by the projection effect. The simulation targets the M87 cluster

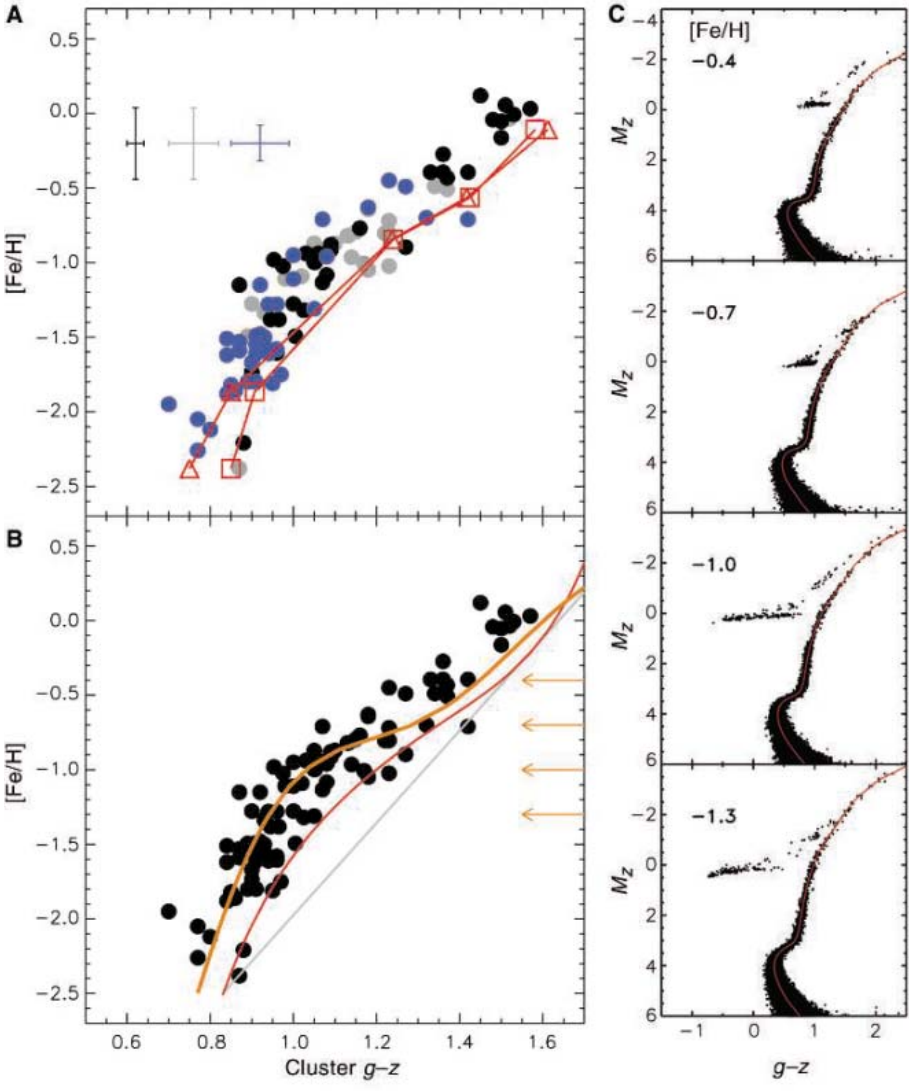

Fig. 1. Correlation between iron abundance [Fe/H] and $g - z$ for globular clusters in our Galaxy and two large elliptical galaxies, M49 and M87. (**A**) The 40 low-extinction ($E_{B-V} < 0.3$) Galactic clusters (blue), 33 M49 and M87 clusters with ACS (the Advanced Camera for Surveys) Virgo Cluster Survey photometry (black), and 22 M49 and M87 clusters with SDSS (Sloan Digital Sky Survey) photometry (gray) were obtained from (*8*). The typical errors have been estimated from references in (*8*) and shown with the same color code. In order to take into account the nonsolar α element (O, Mg, Si, S, Ca, and Ti) abundance of clusters, the α element with respect to iron, [α/Fe] = 0.3 models in (*32*) are used to amend [Fe/H]. Simple stellar population models for 13-Gy clusters are overlaid. Red squares and triangles represent the predictions from (*19*) and (*20*), respectively. (**B**) The identical observed data in (A) are shown. Our equivalent 13-Gy population model (thick orange line) with the finer grid spacing (Δ[Fe/H] = 0.1) is overlaid. The thin red line is for the model without inclusion of the HB variation. The gray line is the linear connection between $g - z$ values of 0.83 and 1.70, which assists in estimating the degree of the departure from the linearity. Arrows denote [Fe/H] values for which the color-magnitude diagrams are shown in (C). (**C**) Synthetic color-magnitude diagrams for 13-Gy clusters with various [Fe/H] values. Red loci are the model isochrones.

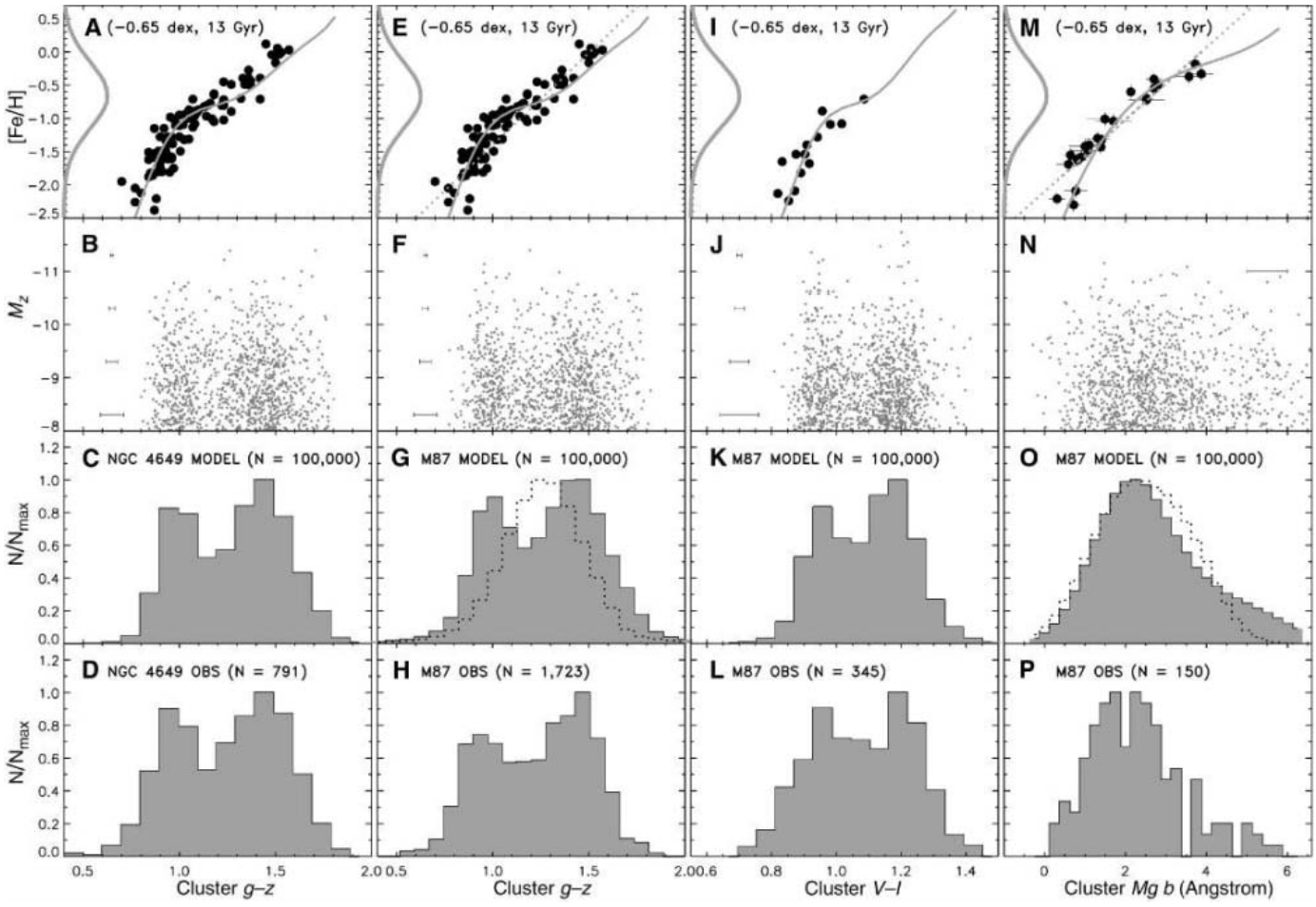

Fig. 2. Monte Carlo simulations of globular cluster color distribution. (**A**) Same as Fig. 1B. The metallicity distribution of 10^5 model clusters is shown along the *y* axis (thick gray line), and their mean metallicity and age are denoted in parenthesis. (**B**) Color-magnitude diagram of 1000 randomly selected model clusters of 13 Gy. Cluster $g - z$ is transformed from [Fe/H] by using the theoretical relation shown in (A). For the integrated *z*-band absolute magnitude, M_z, a Gaussian luminosity distribution ($\langle M_z \rangle = -8.19$ and $\sigma_z = 1.03$) is assumed (*7*). The observational uncertainty in $g - z$ as a function of M_z is taken into account on the basis of the observations (*5*). (**C**) The color histogram of 10^5 model clusters of 13 Gy. (**D**) The observed color histogram for 791 clusters in NGC 4649 (*8*). (**E** to **H**) Same as (A) to (D), but for 1723 clusters in M87 (*8*). The dotted histogram in (G) represents the distribution that is obtained by using a simple linear fit shown by dotted line in (E). (**I** to **L**) Same as (E) to (H), but for the *V* (~5550 Å) − *I* (~8140 Å) color. The data in (I) are from (*33*). The observed histogram is for 345 clusters in M87 (*5*). (**M** to **P**) Same as (E) to (H), but for *Mg b*. The data in (M) are from (*26*). The dotted histogram in (O) represents the distribution that is obtained by using a simple linear fit shown by dotted line in (M). The observed histogram is for 150 clusters in M87 (*25*). The uncertainty of 0.5 Å is assumed (*25*).

system, which has a clear color bimodality (Fig. 2, H and L) (*8*, *11*) and the largest cluster sample with measured metal indices (*25*). The observed *Mg b* distribution is, although not bimodal, highly asymmetric (Fig. 2P), which is often viewed as the sum of two cluster subsystems. Because the absorption indices trace more directly the element abundance than colors do, the model predicts a relatively weaker wavy feature along the [Fe/H]–*Mg b* relation (Fig. 2M), which is in good agreement with the observation (*26*). When the identical [Fe/H] distribution to that in Fig. 2, E and I, is used, the model (Fig. 2O) successfully reproduces the observed *Mg b* histogram that has a broad metal-poor peak with a metal-rich tail (Fig. 2P). For comparison, the color distribution (Fig. 2O) that is obtained by using a simple linear fit to the [Fe/H] versus *Mg b* data is in clear conflict with the observation. We therefore conclude that the projection effect is also at work in the *Mg b* distribution of M87 clusters.

There is a growing body of evidence that the color distributions of globular cluster systems are closely linked to the host galaxy luminosity (*2*–*8*). The number fraction of red clusters and the mean colors of both blue and red clusters increase progressively for more luminous galaxies. We have found that the projection effect can explain these intriguing trends as well. To simulate the color distributions as a function of host galaxy *B*-band luminosity (M_B), we have adopted a $\langle [Fe/H] \rangle_{GC}$ for each cluster system of various M_B on the basis of the empirical $\langle [Fe/H] \rangle_{GC}$-$M_B$ relation (*8*) (Fig. 3D). The resulting color histograms (Fig. 3B) show that, in proceeding to lower luminosities, the distributions are more consistent with being predominantly blue peaked. This is in good accordance with the observations (*8*) (Fig. 3C).

We compared the observed quantities across galaxy luminosity with those obtained from the model histograms. The observed link of the red cluster fraction (Fig. 3E) and the blue and red peak colors (Fig. 3F) to the host galaxy luminosity is well reproduced by means of the projection effect. Interestingly, the zero point for red cluster colors is on average ~0.1 magnitude redder than the observations. This can be explained (Fig. 3, A and F) if red clusters are slightly younger than blue ones (by ~2 Gy) within the current uncertainty in cluster age dating (*9*) or if red clusters with [Fe/H] > −0.5 have an extended blue HB component, as observed in the Galactic counterparts (*27*, *28*).

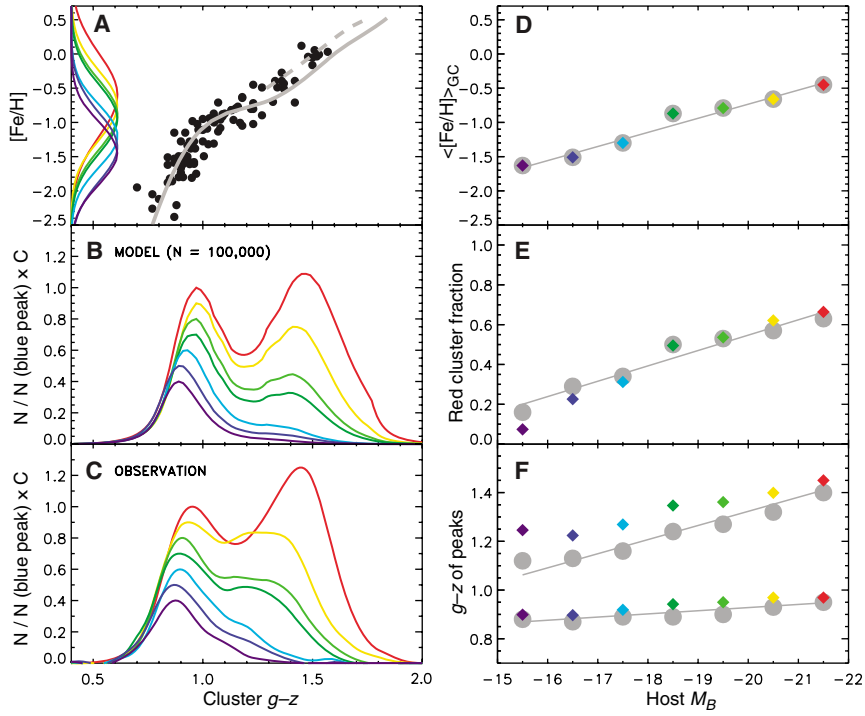

Fig. 3. Monte Carlo simulations of globular cluster color distributions for various host galaxy luminosities. (**A**) Same as Fig. 1B. Various metallicity distributions of 10^5 model clusters are shown along the *y* axis. The values of $\langle[Fe/H]\rangle_{GC}$ are adopted from the observations shown in (D). Both the 11-Gy model and the model with extended blue HB component (30% in number) are given by the dashed line. (**B**) The model color histograms of cluster systems for seven bins of host galaxy magnitude. The histograms are normalized by the number of blue clusters and multiplied by constants, *C*, for clarity. The magnitude bins are 1 magnitude wide and extend from $M_B = -21$ (red, $C = 1.0$) to -15 (purple, $C = 0.4$). (**C**) The observed color histograms of clusters in the same magnitude bins, multiplied by *C*. (**D** to **F**) The observational data—the mean metallicity $\langle[Fe/H]\rangle_{GC}$, the fraction of red clusters, and the peak colors of blue and red clusters—as functions of M_B (*8*) are denoted by large gray circles. The solid line in each panel is the least-squares fit. The simulation results are marked by small diamonds with the same color code as in (A) to (C).

Besides, the observed slope for red clusters is known to be 4.6 times steeper than that for blue clusters (*8*) (Fig. 3F). This is probably because the slope in the [Fe/H]-$g-z$ relation is steeper by such a factor in the metal-rich regime (Fig. 3A). This effect also naturally explains the observed larger color spread of red clusters at given M_B and the larger color ranges of clusters in brighter galaxies. We therefore conclude that the projection effect and the variation of [Fe/H] distribution are the main drivers behind the properties of the cluster color distribution as a function of host galaxy luminosity.

It is now well established that blue and red clusters show differences in spatial distribution and kinematics within individual galaxies (*5*, *10*–*16*). Red clusters appear to be more centrally concentrated and of lower velocity dispersion. The phenomenon can be considered as a close analogy with the aforementioned link between the color distributions and the host galaxy luminosities. Observational evidence (*11*, *25*) indicates higher $\langle[Fe/H]\rangle_{GC}$ toward the galaxy center. If this trend is used in the simulation, then the projection effect naturally causes the red clusters to be more popular toward the galaxy center. Besides, kinematic studies indicate that the systematically lower velocity dispersion of red clusters is simply a consequence of the red cluster number density that is higher in the galaxy center (*16*). Thus, it appears that the differences between blue and red clusters in spatial distribution and kinematics are fully consistent with the projection effect explanation.

It has been a popular view that the presence of two discrete cluster subsystems within individual galaxies is responsible for the color bimodality. Our own Galaxy, which possesses two globular cluster subsystems with different metallicity and kinematics (*29*), has served as a typical example of the case. Whether elliptical galaxies and the Milky Way have an equivalent cluster formation history is an outstanding issue, however. One may argue that the Milky Way is not an archetypal host of the color bimodality found in elliptical galaxies, because the origin of the metal-rich component in the Galactic globular cluster system is apparently more complicated (*29*–*31*). With true metallicity being bimodal, color bimodality would be strengthened further. But the essence of our explanation is that we do not need to invoke two distinct metallicity groups to explain the observed level of color bimodality in elliptical galaxies. Further spectroscopic observations are needed to obtain true metallicity distributions of globular cluster systems in elliptical galaxies from high signal-to-noise spectra.

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Quantum Computation as Geometry

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Quantum computers hold great promise for solving interesting computational problems, but it remains a challenge to find efficient quantum circuits that can perform these complicated tasks. Here we show that finding optimal quantum circuits is essentially equivalent to finding the shortest path between two points in a certain curved geometry. By recasting the problem of finding quantum circuits as a geometric problem, we open up the possibility of using the mathematical techniques of Riemannian geometry to suggest new quantum algorithms or to prove limitations on the power of quantum computers.

Quantum computers have the potential to solve efficiently some problems that are considered intractable on conventional classical computers: The most famous example is Shor's algorithm (*1*) for finding the prime factors of an integer. Despite this great promise, as yet there is no general method for constructing good quantum algorithms, and very little is known about the potential power (or limitations) of quantum computers.

A quantum computation is usually described as a sequence of logical gates, each coupling only a small number of qubits. The sequence of gates determines a unitary evolution U performed by the computer. The difficulty of performing the computation is characterized by the number of gates used by the algorithm, which is said to be efficient if the number of gates required grows only polynomially with the size of problem (e.g., with the number of digits in the number to be factored, in the case of Shor's factoring algorithm).

We developed an alternate approach to understanding the difficulty of implementing a unitary operation U. Suppose that U is generated by some time-dependent Hamiltonian $H(t)$ according to the Schrödinger equation $dU/dt = -iHU$, where i is $\sqrt{-1}$ and with the requirement that at an appropriate final time t_f, $U(t_f) = U$. We characterized the difficulty of the computation by imposing a cost $F[H(t)]$ on the Hamiltonian control, $H(t)$. Following (*2*), we chose a cost function on $H(t)$ that defines a Riemannian geometry on the space of unitary operations. Finding the optimal control function $H(t)$ for synthesizing a desired unitary U then corresponds to finding minimal geodesics of the Riemannian geometry.

We show here that the minimal geodesic distance between the identity operation I and U is essentially equivalent to the number of gates required to synthesize U. This result extends the work in (*2*), where it was shown that the minimal distance provides a lower bound on the number of gates required to synthesize U.

Our result allows the tools of Riemannian geometry to be applied to understand quantum computation. In particular, we can use a powerful tool—the calculus of variations—to find the geodesics of the space. Just as in general relativity, this calculus can be used to derive the geodesic equation, a "force law" whereby the local shape of space tells us how to move in order to follow the geodesics of the manifold.

Intuitively, our results show that the optimal way of solving any computational problem is to "fall freely" along the minimal geodesic curve connecting the identity operation to the desired operation, with the motion determined entirely by the local "shape" of the space. To appreciate this result, consider that once an initial position and velocity are set, the remainder of the geodesic is completely determined by the geodesic equation. This is in contrast with the usual case in circuit design, either classical or quantum, where being given part of an optimal circuit does not obviously assist in the design of the rest of the circuit. Geodesic analysis thus offers a potentially powerful approach to the analysis of quantum computation. However, a caveat to this optimism is that although we know the initial position is the identity operation, we still need to determine the initial velocity in order to find the minimal geodesic; this is not, in general, an easy problem.

Our results can also be viewed as showing that the problem of finding minimal quantum circuits is equivalent to a problem in geometric control theory (*3*), which has had great success in using techniques from the calculus of variations and Riemannian geometry to solve optimal control problems. For example, Khaneja and co-workers (*4*) and others (*5*, *6*) have used geometric techniques to analyze the minimal time cost of synthesizing two-qubit unitary operations using a fixed, two-qubit control Hamiltonian and fast local control.

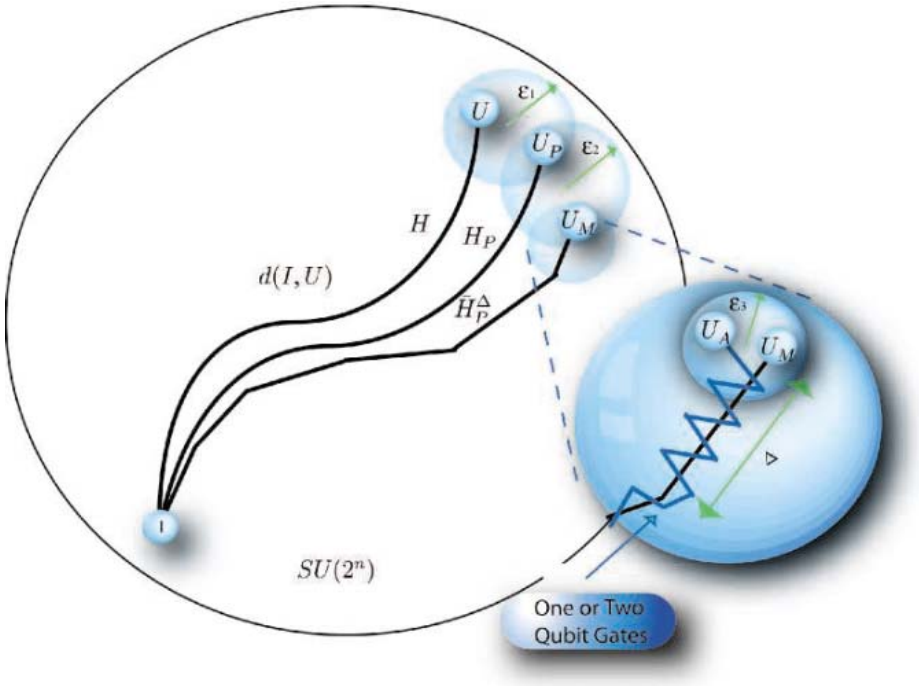

Fig. 1. Schematic of the three steps used to construct a quantum circuit approximating the unitary operation U. The circuit is of a size that is polynomial in the distance $d(I, U)$ between the identity and U. First, we projected the Hamiltonian $H(t)$ for the minimal geodesic path onto one- and two-qubit terms, giving $H_P(t)$. By choosing the penalty p large enough ($p = 4^n$), we ensured the error in this approximation is small, $\varepsilon_1 \leq d(I, U)/2^n$. Next, we broke up the evolution according to $H_P(t)$ into N small time steps of size $\Delta = d(I, U)/N$, and we approximated with a constant mean Hamiltonian $\bar{H}_P^\Delta$ over each step. Finally, we approximated evolution according to the constant mean Hamiltonian over each step by a sequence of one- and two-qubit quantum gates. The total errors, ε_2 and ε_3, introduced by these approximations can be made smaller than any desired constant by choosing the step size Δ sufficiently small: $\Delta = O(1/[n^2 d(I, U)])$. In total, we need $O(n^6 d(I, U)^3)$ quantum gates to approximate U to within some constant error, which can be made arbitrarily small.

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To choose a cost function on the control Hamiltonian $H(t)$, we first write $H(t)$ in terms of the Pauli operator expansion $H = \sum_{\sigma}' h_\sigma \sigma + \sum_{\sigma}'' h_\sigma \sigma$, assuming the following: (i) In the first sum, σ ranges over all possible one- and two-body interactions, that is, over all products of either one or two Pauli matrices acting on n qubits. (ii) In the second sum, σ ranges over all other tensor products of Pauli matrices and the identity. (iii) The h_σ are real coefficients. We then define a measure of the cost of applying a particular Hamiltonian during synthesis of a desired unitary operation

$$F(H) = \sqrt{\sum_{\sigma}' h_\sigma^2 + p^2 \sum_{\sigma}'' h_\sigma^2} \qquad (1)$$

The parameter p is a penalty paid for applying three- and more-body terms; later we will choose p to be large in order to suppress such terms (7).

This definition of control cost leads us to a natural notion of distance in the space $SU(2^n)$ of n-qubit unitary operators with unit determinant. A curve $[U]$ between the identity operation I and the desired operation U is a smooth function U: $[0, t_f] \rightarrow SU(2^n)$, such that $U(0) = I$ and $U(t_f) = U$. The length of this curve can then be defined by the total cost of synthesizing the Hamiltonian that generates evolution along the curve

$$d([U]) \equiv \int_0^{t_f} dt F[H(t)] \qquad (2)$$

Because $d([U])$ is invariant with respect to different parameterizations of $[U]$ (*8*), we can always rescale the Hamiltonian $H(t)$ such that $F[H(t)] = 1$ and the desired unitary U is generated at time $t_f = d([U])$. From now on, we assume that we are working with such normalized curves. Finally, the distance $d(I, U)$ between I and U is defined to be the minimum of $d([U])$ over all curves $[U]$ connecting I and U.

We will show that for any family of unitaries U (implicitly, U is indexed by the number of qubits n), there is a quantum circuit containing a number of gates that is polynomial in $d(I, U)$ and that approximates U to high accuracy. In other words, if the distance $d(I, U)$ scales polynomially with n for some family of unitary operations, then it is possible to find a polynomial-sized quantum circuit for that family of unitary operations. Conversely, the metric we construct also has the property, proved in (*2*), that up to a constant factor, the distance $d(I, U)$ is a lower bound on the number of one- and two-qubit quantum gates required to exactly synthesize U. Consequently, the distance $d(I, U)$ is a good measure of the difficulty of implementing the operation U on a quantum computer.

The function $F(H)$ specified by eq. 1 can be thought of as the norm associated to a (right invariant) Riemannian metric whose metric tensor g has components:

$$g_{\sigma\tau} = \begin{cases} 0 & \text{if } \sigma \neq \tau \\ 1 & \text{if } \sigma = \tau \text{ and } \sigma \text{ is one- or two-body} \\ p^2 & \text{if } \sigma = \tau \text{ and } \sigma \text{ is three- or more-body} \end{cases} \qquad (3)$$

These components are written with respect to a basis for the local tangent space corresponding to the Pauli expansion coefficients h_σ. The distance $d(I, U)$ is equal to the minimal length solution to the geodesic equation, which may be written (*9*) as $\langle dH/dt, K\rangle = i\langle H, [H, K]\rangle$. In this expression, the notation $\langle x, y\rangle$ indicates the inner product of x and y on the tangent space $su(2^n)$ defined by the metric components of eq. 3, the notation $[H, K]$ indicates the matrix commutator, and K is an arbitrary operator. For our particular choice of metric components, this geodesic equation may be rewritten as

$$p_\sigma^2 \dot{h}_\sigma = i \sum_\tau p_\tau^2 h_\tau \tilde{h}_{[\sigma,\tau]} \qquad (4)$$

where $\tilde{h}_{[\sigma,\tau]} = \mathrm{tr}(H[\sigma, \tau])/2^n$ and tr indicates the trace. A particular class of solutions to this equation was studied in (*2*), but understanding the general behavior of the geodesics remains a problem for future research (*10*). There are powerful tools in Riemannian geometry (*11*, *12*) available for the study of minimal length geodesics.

Our goal is to use the optimal control Hamiltonian $H(t)$ to construct explicitly a quantum circuit containing a number of gates that is polynomial in $d(I, U)$ and which approximates U closely. The construction combines three main ideas, which we express through three separate lemmas, before combining them to obtain the result (Fig. 1).

The first lemma shows that the error that arises by simply ignoring the many-body interactions in $H(t)$ can be made small by choosing the penalty p appropriately. We define H_P to be the projected Hamiltonian formed by deleting all three- and more-body terms in the Pauli expansion. The following result is proved in (*13*).

Lemma 1: Let $H_P(t)$ be the projected Hamiltonian obtained from a Hamiltonian $H(t)$ generating a unitary U. Let U_P be the corresponding unitary generated by $H_P(t)$. Then

$$\|U - U_P\| \leq \frac{2^n d([U])}{p} \qquad (5)$$

where $\|x\|$ is the operator norm of x (*14*) and p is the penalty parameter appearing in the definition of the metric. Thus, by choosing p sufficiently large, say $p = 4^n$, we can ensure that $\|U - U_P\| \leq d([U])/2^n$.

Motivated by the preceding lemma, we change our aim from accurately synthesizing U to accurately synthesizing U_P. To do this, we break the evolution according to $H_P(t)$ up into many small intervals, each of length Δ. The next lemma shows that evolution according to the time-dependent Hamiltonian $H_P(t)$ over such a small time interval can always be accurately simulated by a constant mean Hamiltonian, which we denote $\overline{H}_P^\Delta$.

Lemma 2: Let U be an n-qubit unitary generated by applying a time-dependent Hamiltonian $H(t)$ satisfying $\|H(t)\| \leq c$, for some constant c, over a time interval $[0, \Delta]$. Then defining the mean Hamiltonian $\overline{H} \equiv \frac{1}{\Delta}\int_0^\Delta dt H(t)$ we have

$$\|U - \exp(-i\overline{H}\Delta)\| \leq 2(e^{c\Delta} - 1 - c\Delta) = O(c^2\Delta^2) \qquad (6)$$

where $O(x)$ indicates the asymptotic behavior of the function. The proof of this lemma is based on the Dyson operator expansion and is presented in (*13*). To apply this lemma to $H_P(t)$, note that elementary norm inequalities and the observation $F[H_P(t)] \leq 1$ imply that $\|H_P(t)\| \leq (3/\sqrt{2})nF[H_P(t)] \leq (3/\sqrt{2})n$ (*15*). Lemma 2 implies that over a time interval Δ, we have

$$\|U_P^\Delta - \exp(-i\overline{H}_P^\Delta\Delta)\| \leq 2\left[e^{3/\sqrt{2}n\Delta} - (1 + \frac{3}{\sqrt{2}}n\Delta)\right] = O(n^2\Delta^2) \qquad (7)$$

where U_P^Δ is the evolution generated by $H_P(t)$ over the time interval Δ, and $\overline{H}_P^\Delta$ is the corresponding mean Hamiltonian.

Our third and final lemma shows that evolution according to a time-independent Hamiltonian H containing only one- and two-body terms can be very accurately simulated by using a number of quantum gates that is not too large.

Lemma 3: Suppose H is *an* n-qubit two-body Hamiltonian whose Pauli expansion coefficients satisfy $|h_\sigma| \leq 1$. Then there exists a unitary U_A, satisfying

$$\|e^{-iH\Delta} - U_A\| \leq c_2 n^4 \Delta^3 \qquad (8)$$

that can be synthesized using at most $c_1 n^2/\Delta$ one- and two-qubit gates, where c_1 and c_2 are constants.

This result follows from standard procedures for simulating quantum evolutions using quantum gates [(*16*) chap. 4], and it is proved in (*13*). The average Hamiltonian $\overline{H}_P^\Delta$ provided by lemma 2 satisfies the assumptions of lemma 3, because the Pauli expansion coefficients of $H_P(t)$ satisfy $|h_\sigma| \leq 1$ for all times.

To integrate lemmas 1 to 3, suppose $H(t)$ is the time-dependent normalized Hamiltonian generating the minimal geodesic of length $d(I, U)$. Let $H_P(t)$ be the corresponding projected

Hamiltonian, which generates U_P and satisfies $\|U - U_P\| \leq d(I, U)/2^n$, as guaranteed by lemma 1, and where we have chosen $p = 4^n$ as the penalty. Now divide the time interval $[0, d(I, U)]$ up into a large number N of time intervals each of length $\Delta = d(I, U)/N$. Let U_P^j be the unitary operation generated by $H_P(t)$ over the jth time interval, where j is an integer. Let U_M^j be the unitary operation generated by the corresponding mean Hamiltonian. Then lemma 2 implies that:

$$\|U_P^j - U_M^j\| \leq 2[e^{3\sqrt{2}n\Delta} - (1 + \frac{3}{\sqrt{2}}n\Delta)] \quad (9)$$

Lemma 3 implies that we can synthesize a unitary operation U_A^j using at most $c_1 n^2/\Delta$ one- and two-qubit gates and satisfying $\|U_M^j - U_A^j\| \leq c_2 n^4 \Delta^3$.

Putting all these results together and applying the triangle inequality repeatedly, we obtain

$$\|U - U_A\| \leq \|U - U_P\| + \|U_P - U_A\| \quad (10)$$

$$\leq \frac{d(I,U)}{2^n} + \sum_{j=1}^{N} \|U_P^j - U_A^j\| \quad (11)$$

$$\leq \frac{d(I,U)}{2^n} + \sum_{j=1}^{N} \left(\|U_P^j - U_M^j\| + \|U_M^j - U_A^j\| \right) \quad (12)$$

$$\leq \frac{d(I,U)}{2^n} + 2\frac{d(I,U)}{\Delta} \times \left[e^{(3/\sqrt{2})n\Delta} - \left(1 + \frac{3}{\sqrt{2}}n\Delta\right) \right] + c_2 d(I,U) n^4 \Delta^2 \quad (13)$$

Provided we choose Δ to scale at most as $1/[n^2 d(I, U)]$, we can ensure that the error in our approximation U_A to U is small, and the number of gates scales as $n^6 d(I, U)^3$.

Summing up, we have the following theorem (*17*): Using $O(n^6 d(I, U)^3)$ one- and two-qubit gates, it is possible to synthesize a unitary U_A satisfying $\|U - U_A\| \leq c$, where c is any constant (e.g., $c = 1/10$).

Our results demonstrate that, up to polynomial factors, the optimal way of generating a unitary operation is to move along the minimal geodesic curve connecting I and U. Because the length of such geodesics also provides a lower bound on the minimal number of quantum gates required to generate U, as shown in (*2*), the geometric formulation offers an alternate approach, which may suggest efficient quantum algorithms or provide a way of proving that a given algorithm is indeed optimal.

It would, of course, be desirable to completely classify the geodesics of the metric we constructed. An infinite class of such geodesics has been constructed in (*2*) and is shown to have an intriguing connection to the problem of finding the closest vector in a lattice. A more complete classification of the geodesics could provide major insight on the potential power of quantum computation.

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Effects of Solar Flares on the Ionosphere of Mars

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All planetary atmospheres respond to the enhanced x-rays and ultraviolet (UV) light emitted from the Sun during a flare. Yet only on Earth are observations so continuous that the consequences of these essentially unpredictable events can be measured reliably. Here, we report observations of solar flares, causing up to 200% enhancements to the ionosphere of Mars, as recorded by the Mars Global Surveyor in April 2001. Modeling the altitude dependence of these effects requires that relative enhancements in the soft x-ray fluxes far exceed those in the UV.

Sudden changes in the Sun's photon radiation and in the particles and fields of its solar wind reach Earth in about 8 min and a few days, respectively. These enhanced sources of energy cause sudden atmospheric disturbances and the auroral displays associated with longer lived geomagnetic storms. The recent availability of spacecraft orbiting other planets has enabled studies of such effects on other worlds. A mass ejection from the Sun's corona in early November 2000 caused auroras on Earth, Jupiter, and Saturn during its month-long traverse through the solar system, providing a specific challenge to models that track solar wind density and magnetic field enhancements (*1*). Increased x-ray emissions were observed from Jupiter and Saturn in November 2003 and January 2004, respectively, shortly after solar flares, thereby demonstrating the Sun's control of nonauroral x-ray emission from giant planets (*2*, *3*). However, the direct response of another planetary atmosphere to solar flare photons, e.g., suddenly enhancing its ionosphere, has not been seen. Here, we report such an effect in the ionosphere of Mars.

Ions and electrons in a planet's ionosphere are produced by the photoionization of neutral

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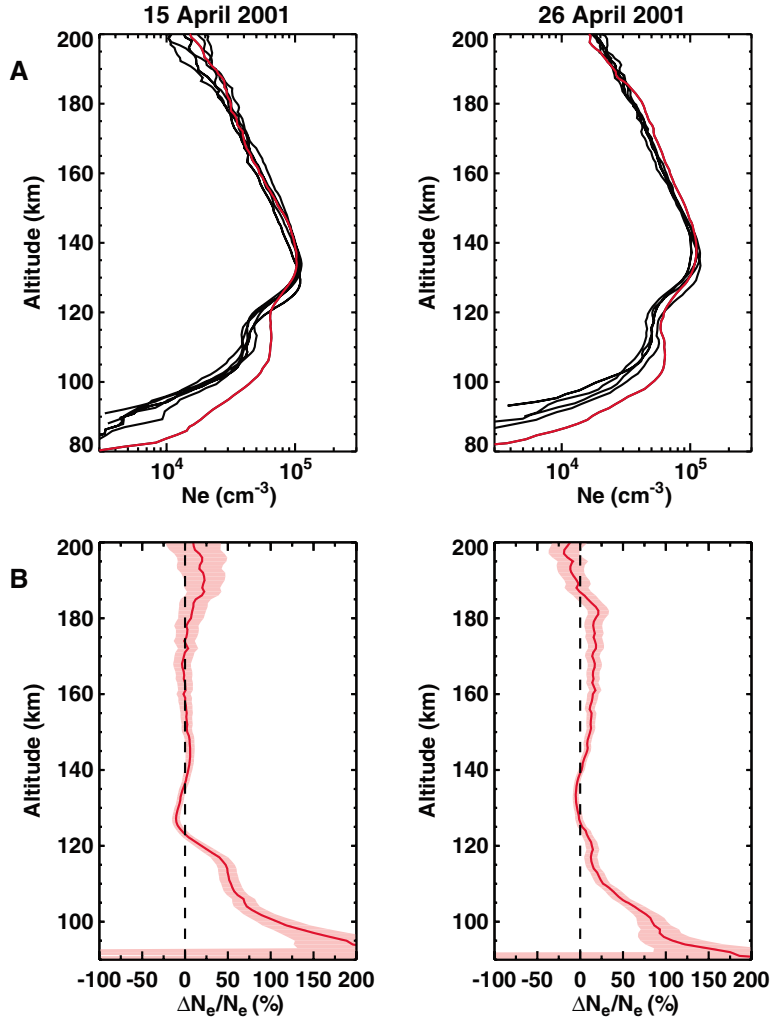


Fig. 1. (**A**) Electron density profiles on Mars obtained for 15 April and 26 April 2001. Measurement uncertainty is several thousand electrons/cm^3, and thus the two profiles in red [14:15 and 13:16 universal time (UT), respectively] show statistically significant departures at low altitudes because of solar flares. On 15 April, there were five MGS profiles before the flare, at 02:28, 06:23, 08:21, 10:19, and 12:17 UT, and none after the flare; on 26 April, preflare profiles were available at 09:20 and 11:18 UT, and postflare, at 17:11 and 19:09 UT. (**B**) Percentage differences between the flare-affected profiles and the averages of the other profiles on each day. The shadings give the 1-σ standard error in the relative change in N_e.

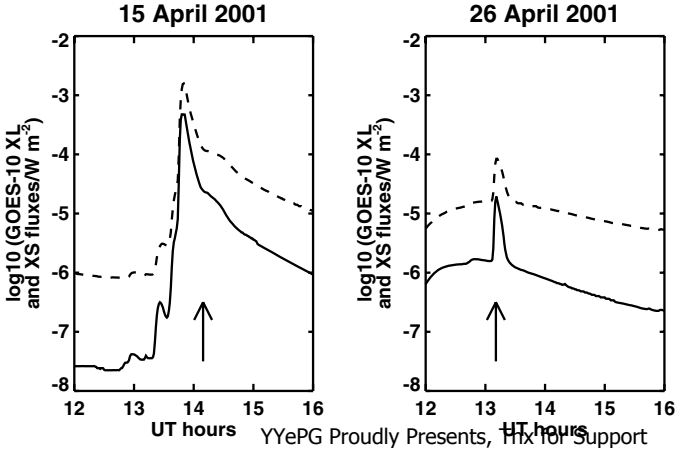
species by solar extreme ultraviolet (EUV) and x-ray photons (*4*, *5*). The ionizing solar flux and thus the ionosphere are variable on many time scales. The most important is the 11-year solar cycle, whereas the shortest is the solar flare, an impulsive emission of photons that peaks within minutes and takes tens of minutes, and perhaps hours, to decay to preflare levels. Flares affect Earth's atmosphere and radio propagation controlled by its ionosphere. Such effects have been used historically as proxies to deduce how the Sun's EUV and x-rays changed during a flare (*6*, *7*).

Artificial satellites passing by or orbiting Mars are required to conduct radio soundings of its ionosphere. The 443 published measurements obtained by U.S. and Soviet probes between 1965 and 1980 led to a basic understanding of the structure of its electron density profile (*5*, *8*, *9*). Depending on solar zenith angle, the martian ionosphere has a main peak between 120 and 140 km produced by ultraviolet photons in the wavelength range from 200 to 800 Å. A lower peak, or ledge, at 90 to 110 km is produced by x-rays (<100 Å). In 1998, the Mars Global Surveyor (MGS), with its radio science experiment (*10*), was inserted into orbit around Mars. By 2001, MGS made 1867 measurements of the martian ionosphere, enabling investigation of ionospheric variability and its causes (*9*). MGS transmits a 3.6-cm-wavelength radio signal to Earth, and, as it passes behind or emerges from the far side of the planet, the propagation is perturbed by the neutral atmosphere and the ionosphere of Mars. The observable effect is a Doppler shift in the frequency of the signal received on Earth, and vertical profiles of electron density can be retrieved from such data.

Six MGS profiles of electron density versus height, $N_e(h)$, were measured on 15 April 2001, and five were measured on 26 April 2001 (Fig. 1). All the profiles refer essentially to the same latitude (84°N) and local time (08:40 LT) on Mars but were made at different longitudes at ~2-hour intervals. All profiles show a stable pair of layers: the main one near 130 km and a secondary peak between 105 and 110 km. Day-to-day variability is 5 to 7% at the main peak and ~10% at the lower peak (*8*). In one case on each day, the electron densities at and below the secondary peak are enhanced by 50 to 200%. We have traced these dramatic changes to x-rays from solar flares that occurred within minutes of each observation (Fig. 2).

The Geostationary Operational Environmental Satellites (GOES) (*11*) in orbit about Earth make continuous observations of solar x-rays. The X14.4 flare of 15 April was the second strongest in 2001, whereas the M7.8 flare of 26 April was far more moderate. The Sun-Earth-Mars angle was only ~26° at these times, and so it is reasonable to apply the solar irradiance (photon flux versus wavelength) measured at Earth to Mars with a 4.5-min

Fig. 2. Solar x-ray fluxes on 15 and 26 April 2001 measured by the GOES spacecraft at Earth for two wavelength bands (*11*): XS (0.5 to 3 Å), solid line, and XL (1 to 8 Å), dashed line. The peak fluxes at Earth occurred at 13:50 UT and 13:10 UT, respectively. The solar fluxes incident upon Mars at the times of the flare-affected profiles in Fig. 1 are marked by arrows.



delay. Thus, the peak fluxes on 15 and 26 April reached Mars ~20 min and ~90 s, respectively, before the profiles highlighted (Fig. 1).

Although GOES x-rays can be enhanced by orders of magnitude during a flare, they are too energetic ("hard") to produce the ionospheric enhancements shown. They penetrate to altitudes around 60 km, where unambiguous MGS measurements of electron density are not available. Very large relative increases in N_e must have occurred suddenly at these low heights. At the altitudes of the enhancements observed by MGS, ionization is caused by softer x-rays in the 18- to 50-Å range (*9*, *12*), a wavelength region not measured by GOES. At about 110 km, production by photons ($P = F_s\sigma\eta N$) equals chemical loss ($L = \alpha N_e^2$), where F_s is the effective flux of solar photons that cause ionization, σ is the cross section for ionization of CO_2, η is the number of ion-electron pairs created per x-ray photon absorbed, N is the concentration of CO_2, α is the dissociative recombination coefficient for O_2^+ and electrons, and N_e is the electron density. Thus, for an observed flare-induced electron density (N_e^f) 1.5 times the preflare value (N_e^o) at 110 km, the solar flare's ionizing flux (F_s^f) with respect to its value before the flare (F_s^o) would be

$$F_s^f/F_s^o = (N_e^f/N_e^o)^2 = (1.5)^2 = 2.25 \quad (1)$$

Increases by factors of 2 to 3 in the Sun's soft x-ray flux during a flare are well within observed variabilities (*13*).

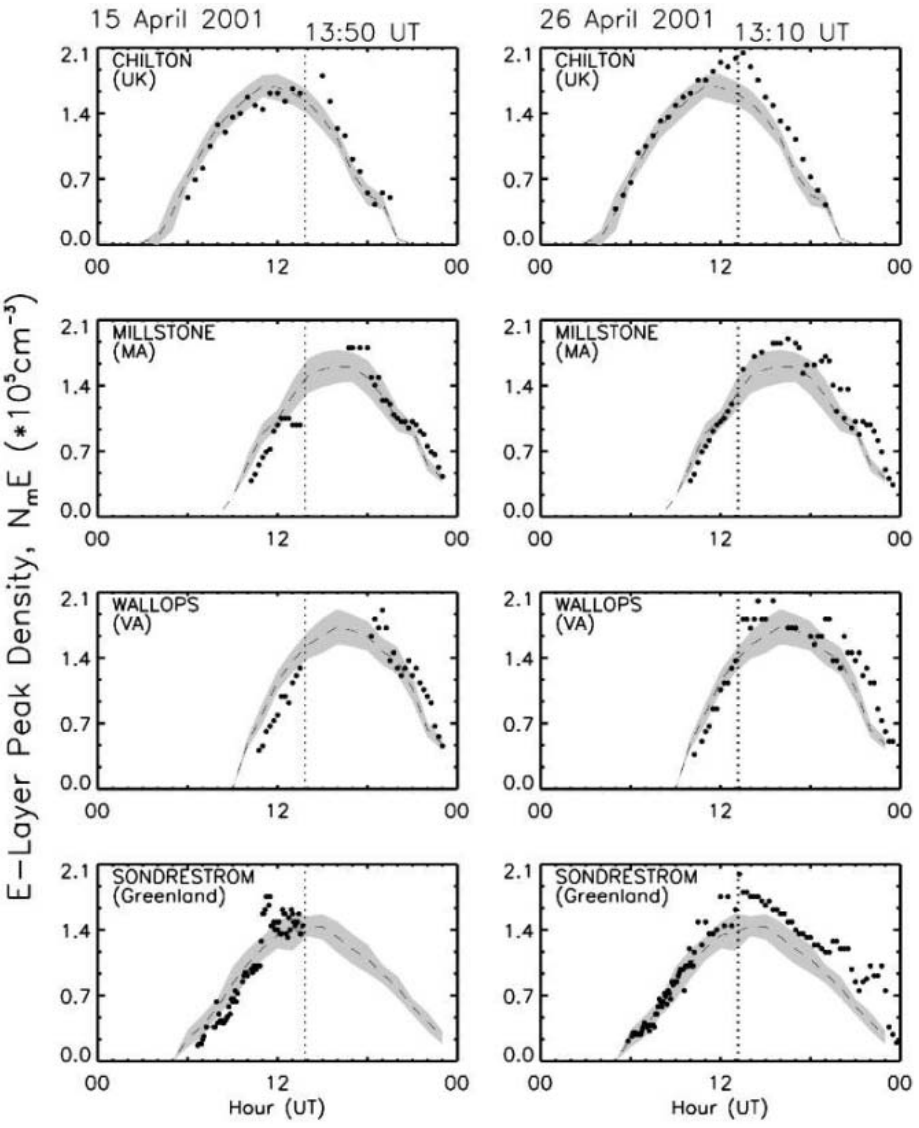


Fig. 3. The maximum electron densities of the Earth's E layer (N_mE) for 15 April and 26 April 2001. For each station, the monthly mean pattern is given by the dashed lines, their standard deviations (1 σ) by the shading, and individual data points by dots (scaled to 0.1 MHz, giving ~10% uncertainty). The vertical dotted lines show the times of peak flare fluxes. At the highest latitude ionosonde station (Sondrestrom, Greenland), there is additional preflare E layer variability caused by auroral activity and sporadic E layers.

The Solar and Heliospheric Observatory (SOHO) spacecraft also observes the Sun from Earth orbit, but it only had data for 15 April. Those data show that at the time of the MGS measurements EUV fluxes (260 to 340 Å) were enhanced by only ~10%, whereas fluxes integrated over the broad wavelength range from 1 to 500 Å (soft x-rays plus EUV) increased by ~50%. This trend suggests greater enhancements in the 1- to 50-Å range (soft x-rays only) (*14*), consistent with the observed changes in the martian ionosphere being confined to lower heights.

The responses of the martian ionosphere were similar for these two flares despite their very different peak fluxes. This is because the postflare $N_e(h)$ profile for the weaker event (26 April) was measured just 90 s after the peak x-ray flux, whereas for the far stronger event on 15 April the MGS observation was made 20 min into the flare's decay phase. The GOES fluxes of hard x-rays were of comparable magnitude at these times, and thus it is reasonable that the soft x-rays fluxes were also similar.

To show that such flares have consequences on Earth, we searched for terrestrial measurements made at ionosonde sites that were in daylight at the times of both flares. An ionosonde (*15*) is essentially a radar for electrons that transmits frequencies in the 1- to 30-MHz range and records the time delays of echoes reflected by the ionosphere. Earth's ionosphere has multiple layers, with a main high-altitude peak near 300 km (called the F layer) produced primarily by EUV, an E layer near 100 km produced by soft x-rays and EUV, and a D layer near 70 km produced by hard x-rays. For comparison with Mars's secondary peak, the terrestrial E layer is the most appropriate one because of the wavelengths that produce it and because the F layer is heavily influenced by transport processes. We plotted the E layer maximum electron density (N_mE) versus LT in Fig. 3. The four data sets span high latitudes (Sondrestrom, Greenland) to midlatitudes (Chilton, UK; Millstone Hill, MA; and Wallops Island, VA). The solar zenith angle at Sondrestrom for these flares was about 61°, somewhat comparable to the 72° at the MGS high-latitude observing location on Mars.

The flare of 15 April was so severe that these instruments were unable to observe the ionospheric layers. Enhanced electron densities in the D layer produced by the hard x-rays caused absorption of the radio waves transmitted, thus preventing soundings of the overlying E and F layers. If an ionosonde had been operating on Mars, similar effects would have occurred there as well. This D layer absorption was so severe on Earth that observations were not possible for the rest of the day at Sondrestrom, for 3 hours at Millstone Hill and Wallops Island, and for only an hour at Chilton. When observations resumed at the latter sites, N_mE was higher than might be expected had the flare not occurred. Thus,

the major signature of the 15 April flare on Earth was the elimination of reliable ionospheric data.

More continuous data sets exist for the 26 April flare, and they show the expected enhancements of the E layer electron densities by the flare. At Sondrestrom, the site most appropriate for comparisons with MGS at Mars, the electron density increased by ~45%. Because the E layer is caused by both EUV and soft x-rays and the EUV changed only slightly, the Sondrestrom results must be due to the more-than-double effective ionizing fluxes, in agreement with Eq. 1 applied at Mars. We conclude that these two flares produced near-simultaneous enhancements in the ionospheres of Earth and Mars and that the greater relative increase at lower altitudes in Mars' $N_e(h)$ is consistent with the typical flare spectrum of greater relative flux increases at shorter wavelengths. The N_e increase at Mars is also consistent with the enhancement at its corresponding site on Earth.

The detection of solar flare effects in the martian ionosphere has important consequences. Previous observations and modeling of the responses of planetary ionospheres to changes in solar flux have generally compared solar maximum and minimum conditions. Varying solar fluxes also modify the neutral atmosphere, and thus ionospheric changes result from two highly coupled processes. Although simulations can separate the dependence on each of these parameters, validation from observations over a solar cycle cannot. The observations presented here decouple changes in photon flux due to a flare from far slower changes in the neutral atmosphere, thereby providing a way to constrain photochemistry on two planets simultaneously. This is particularly important for x-ray photons that carry energy far above that needed to ionize an atom or molecule. In such cases, the electron liberated by ionization has so much extra energy that it ionizes other atoms and molecules via collisions. This secondary ionization by photoelectrons is an amplification effect that needs validation throughout the solar system. For Venus, Earth, and Mars, where ionospheric layers have identical end product ions (O_2^+), solar flares offer tests for both primary and secondary ionization coupled to an identical chemical loss mechanism. Calculations using the same solar flare input thus provide constraints not possible at a single planet.

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Anthropogenic and Natural Influences in the Evolution of Lower Stratospheric Cooling

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Observations reveal that the substantial cooling of the global lower stratosphere over 1979–2003 occurred in two pronounced steplike transitions. These arose in the aftermath of two major volcanic eruptions, with each cooling transition being followed by a period of relatively steady temperatures. Climate model simulations indicate that the space-time structure of the observed cooling is largely attributable to the combined effect of changes in both anthropogenic factors (ozone depletion and increases in well-mixed greenhouse gases) and natural factors (solar irradiance variation and volcanic aerosols). The anthropogenic factors drove the overall cooling during the period, and the natural ones modulated the evolution of the cooling.

The global lower stratosphere—the region of the atmosphere from ~12 to 22 km above the surface—has cooled substantially over the past two decades (*1*–*5*). The difference in temperature between 2000 and 1979 has been ascribed mainly to ozone depletion and increases in well-mixed greenhouse gases (*4*, *6*–*10*). Observations indicate that the decrease in temperature was steplike rather than a steady decline (*1*, *3*). Although the overall trend in temperature has been modeled previously (*5*, *9*, *10*), the steplike structure and the evolution of the cooling pattern in the observed global temperature time series has not been explained in terms of specific physical causes, whether these be external forcing and/or internal variability of the climate system. Thus, attribution of the unusual cooling features observed during the 1980s and 1990s has yet to be addressed, along with potential implications for the future.

We used a coupled atmosphere-ocean model (*11*–*13*) to demonstrate that the complex space-time pattern of the lower stratospheric temperature anomalies is a consequence of the combined temporal changes in natural forcings [solar irradiance (*14*) and volcanic aerosols (*15*)] and anthropogenic forcings [well-mixed greenhouse gases (*16*), stratospheric (*17*) and tropospheric ozone (*18*), tropospheric aerosols (*18*), and land use (*13*)].

We performed five separate experiments to investigate the contributions of different forcing mechanisms to changes in lower stratospheric temperature: (i) natural plus anthropogenic (AllForc), (ii) natural (Nat), (iii) well-mixed greenhouse gases (Wmgg), (iv) well-mixed greenhouse gases plus stratospheric and tropospheric ozone (WmggO3), and (v) anthropogenic (Anth; that is, WmggO3 plus tropospheric aerosols and land-use change). For each case, an ensemble of simulations was performed. Individual ensemble members started from different points of a long control simulation with a fixed preindustrial (1860) atmospheric composition and were then integrated from 1861 through 2003. There were five ensemble members for (i);

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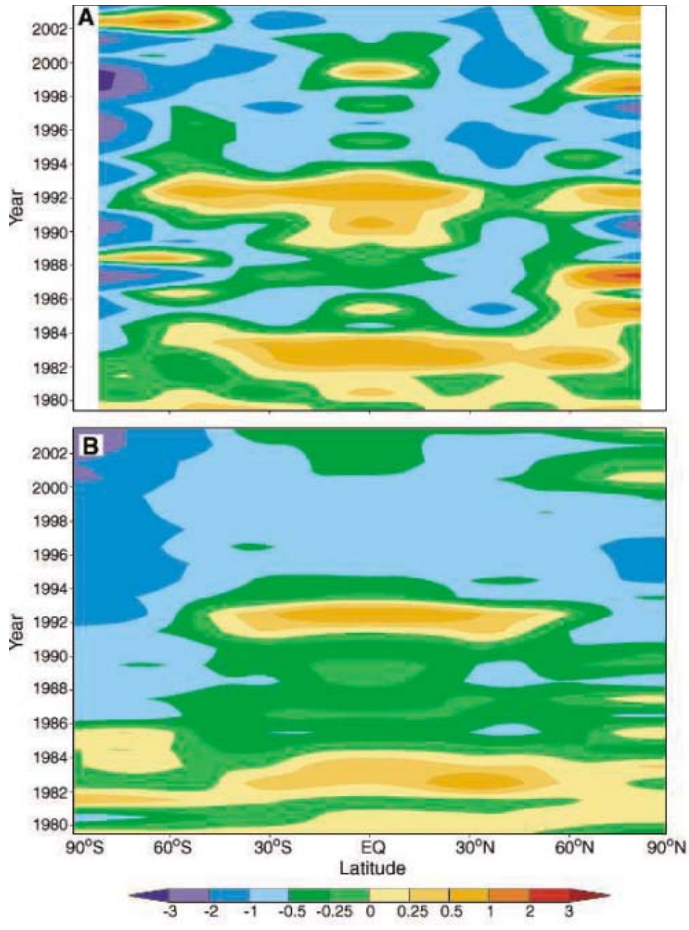

Fig. 1 (left). Zonally and annually averaged temporal evolution (1979–2003) of lower stratospheric temperature (T4) anomalies (relative to 1979–1981 averages). EQ, equator. **(A)** Satellite observations (*19*). **(B)** AllForc model simulated response to natural and anthropogenic forcings. Model results are altitude-weighted as in (*5*), using the T4 weighting function.

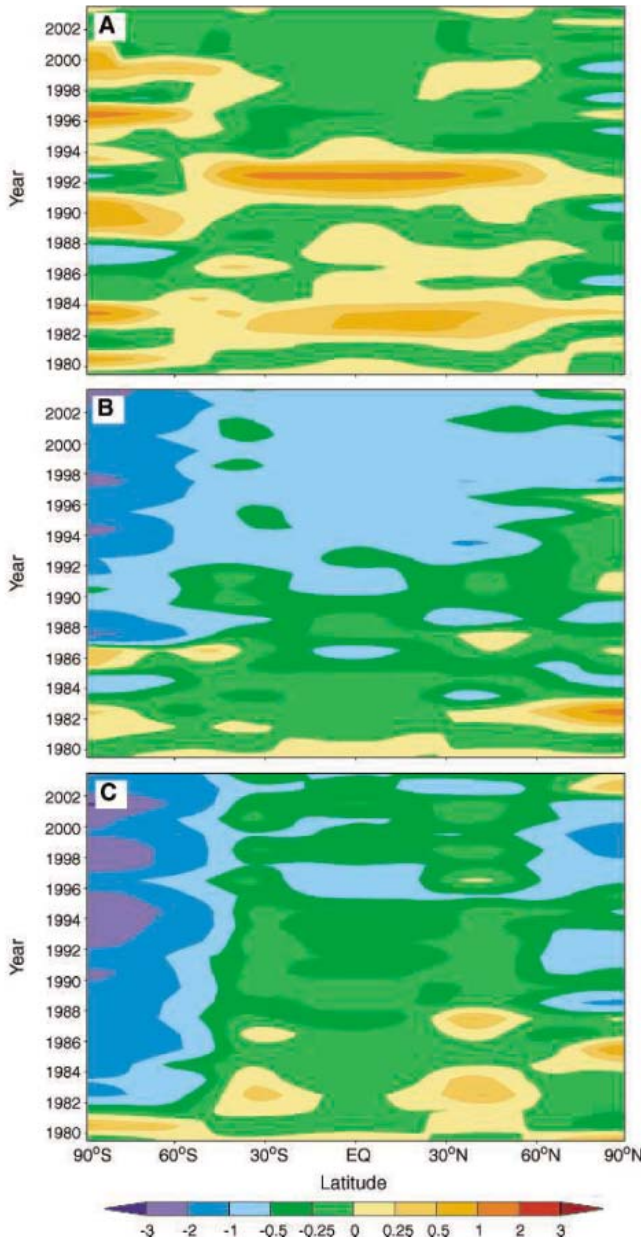

Fig. 2 (right). Ensemble-mean simulated evolution (1979–2003) of the zonally and annually averaged temperature (T4) anomalies (relative to the respective 1979–1981 averages) for **(A)** Nat, **(B)** WmggO3, and **(C)** Anth forcing cases. Model results are altitude-weighted as in (*5*), using the T4 weighting function.

(ii) to (v) comprised three members each, starting from the same initial conditions as three of the AllForc integrations. The observed lower stratospheric temperatures (1979–2003) were from measurements (referred to as T4) made by channel 4 of the Microwave Sounding Unit on the National Oceanic and Atmospheric Administration's satellites (*19*). Synthetic T4 values were computed from the simulated temperature profiles as described in (*5*). The observed and simulated T4 results are expressed as annual mean temperature anomalies, relative to their respective 1979–1981 averages.

The observed time evolution of the zonally and annually averaged T4 temperature anomalies (Fig. 1A) illustrates the transient warming in ~1982–1984 and ~1991–1993 after the El Chichon (1982) and Pinatubo (1991) volcanic eruptions. This warming resulted from the radiative effects of the stratospheric sulfate aerosols formed after the eruptions (*4*, *9*, *10*). After the decay of the volcanic aerosols (~2 years after eruption), the warming ceased, and the lower stratosphere in the 50°N-50°S domain cooled substantially relative to the pre-eruption period. After the steplike transition in ~1985, the observed T4 values were relatively stable until the Pinatubo eruption. The decay of the Pinatubo-related warming was followed by a second steplike cooling in T4 (~1994), and the anomaly remained approximately steady thereafter. The signature of the quasi-biennial oscillation (QBO) in temperature, a natural mode of climate variation (*20*), was clearly manifested at the equator. There was large dynamical variability at high latitudes, especially in the Northern Hemisphere (*4*, *21*).

The AllForc simulation (Fig. 1B) captures the primary features of the observed T4 anomalies, including the El Chichon and Pinatubo warming anomalies; the two steplike cooling transitions; and the ensuing prolonged, quasi-steady periods (~1985–1990 and ~1994–2000). The simulated evolution is spatially more homogeneous and exhibits less cooling than do observations made in the 1980s; the widespread extent and increased cooling in the 1990s is broadly consistent with observations. As in the observations, the model shows both cooling and substantial dynamical variability poleward of 50° (*4*, *6*–*8*). The model lacks a QBO and hence does not replicate the observed periodicity of T4 near the equator.

The simulated influences due to the key forcings are illustrated in Fig. 2. As expected, Nat shows a large stratospheric warming after El Chichon and Pinatubo, but no overall trend (Fig. 2A). In contrast, WmggO3 and Anth (Fig. 2,

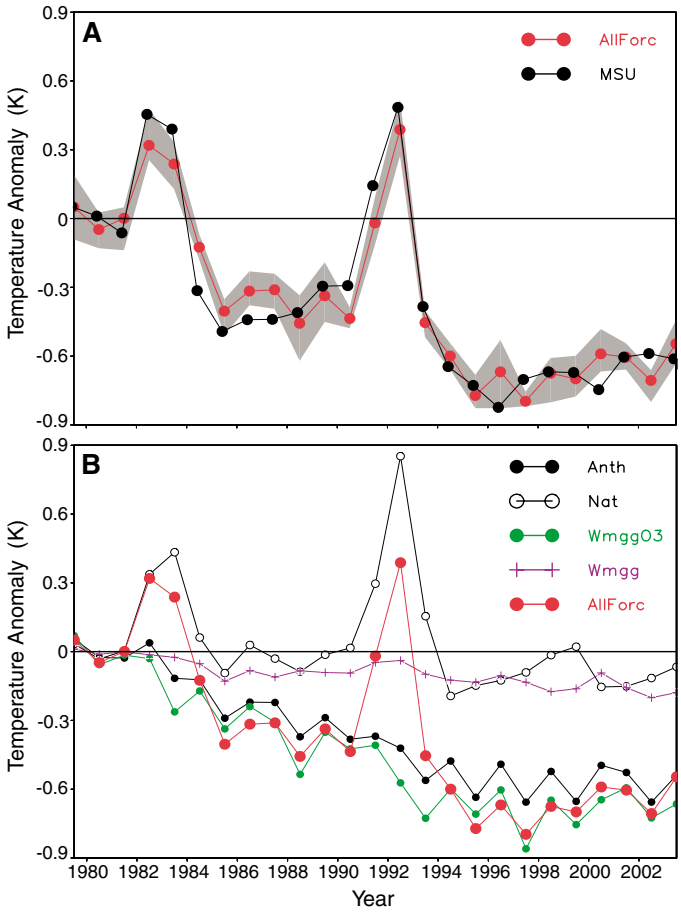

Fig. 3. (**A**) Model-simulated ensemble-mean (AllForc, red curve) and Microwave Sounding Unit (MSU, black curve) satellite observations (*19*) of the globally and annually averaged temperature (T4) anomalies over 1979–2003 (relative to their respective 1979–1981 averages). The gray shading denotes the range of the five-member ensemble simulations and is a measure of the simulated internally generated variability of the climate system. (**B**) Model-simulated ensemble mean of the globally and annually averaged temperature (T4) anomalies (relative to the respective 1979–1981 averages) for the AllForc, Nat, Wmgg, WmggO3, and Anth radiative forcing cases, respectively.

B and C) exhibit a progressive greenhouse gas–dominated cooling of the global stratosphere (*4*–*10*, *22*), with more cooling in the 1990s than in the 1980s. Anth generally has less cooling than WmggO3 at low latitudes. This difference is essentially due to solar warming by anthropogenic upper tropospheric aerosols in Anth (*18*, *23*), with the effect implicitly sampled by the T4 weighting function (*3*–*5*). The increased cooling in AllForc (Fig. 1B) during ~1994–2000 arises primarily from Anth (Fig. 2C), together with a smaller contribution from Nat (Fig. 2A). In contrast, during ~1985–1990, Nat in some domains slightly offsets the Anth cooling (Fig. 2, A and C). Clearly, both Anth and Nat are needed to explain the AllForc evolution and to account for the observed space-time T4 pattern (Fig. 1A).

Figure 3A illustrates that the AllForc simulation successfully captures the complex observed time evolution of the global and annual mean T4 anomalies. The amplitudes of the observed and simulated cooling are very similar, both for the first "step" (1985–1990 minus 1979–1981; results are –0.40 and –0.38 K, respectively) and the second "step" (1994–2000 minus 1985–1990; results are –0.32 and –0.29 K, respectively). The model also replicates the relatively steady behavior of the observed T4 anomalies over ~1994–2000 and the slight temperature increase between the late 1990s and 2003. These results enhance our confidence in the reliability of the model and the applied forcings. Externally forced changes in AllForc are large relative to the model's internally generated variability (Fig. 3A).

In Fig. 3B, the comparison of WmggO3 and Wmgg shows that the overall lower stratospheric temperature decline is driven primarily by the depletion of ozone, and to a lesser extent by the increase in well-mixed greenhouse gases. Although the T4 Anth anomaly is less than WmggO3 because of the previously mentioned solar heating by anthropogenic aerosols in the upper tropsophere (see also Fig. 2, B and C), both exhibit a gradual increase of cooling until the mid- to late 1990s. In contrast, the global average Nat lacks an overall global mean cooling trend but contributes to the AllForc cooling in both decades and is comparable to Wmgg in specific nonvolcanic years.

In the first steplike cooling transition in ~1985, the changes in well-mixed gases and ozone produce an increase in the cooling anomaly of Anth relative to the pre-eruption value (Figs. 2, B and C, and 3B). In contrast to Nat, there is a distinctly increasing cooling of Anth over 1985–1990 (Fig. 3B). During the ~2-year presence of volcanic aerosols, the surface-troposphere system cools; after the aerosols decay, the surface and troposphere temperatures recover, but do so slowly owing to the thermal inertia of the oceans (*9*, *10*, *24*). The cooler surface and troposphere reduce the upwelling long-wave radiation flux, resulting initially in a slight cooling anomaly of the lower stratosphere in Nat. Added to this is the effect of solar cycle variations, yielding decreases in irradiance during the mid-1980s (*1*, *14*). The net result is an approximately steady AllForc anomaly over ~1985–1990 (Figs. 1B and 3B).

The second steplike cooling transition in ~1994 is followed by another near-steady anomaly period (~1994–2000; Fig. 3B). The sharp decline in Anth relative to the pre-eruption value is due to WmggO3 (Figs. 2, B and C, and 3B). In contrast to 1985–1990, stratospheric ozone depletion in the 1990s is easily the principal contributor to the Anth and AllForc cooling. This depletion levels off during the mid- to late 1990s, resulting in a near-flattening of the WmggO3 and Anth anomalies. After ~1998, the cooling anomaly in WmggO3 decreases slightly, consistent with a lesser ozone depletion. The Nat cooling contribution again comprises a post–volcanic-aerosol cooling of the surface and troposphere as in the mid-1980s, plus the effect of another minimum in the solar cycle (*14*). In the mid-1990s, the Nat cooling (Fig. 2A), together with Anth (Fig. 2C), yields a substantial AllForc cooling (Figs. 1B and 3B). By the mid- to late 1990s, the Nat cooling contribution diminishes as the surface-troposphere system recovers from the volcanic cooling and the solar cycle proceeds to its next maximum (*14*). Thus, the deceleration of the AllForc cooling in the mid- to late 1990s (Figs. 1B and 3B) is traceable to stratospheric ozone changes coupled with natural influences.

The two steplike temperature transitions, followed by relatively steady periods (Figs. 1 and 3A), are thus a consequence of both anthropogenic and natural factors. The anthropogenic cooling influence becomes an important global feature by the late 1980s and a dominant one in the 1990s (Figs. 2, B and C, and 3B). Although the effect of volcanic aerosols on the transient warming in the lower stratosphere is known (*4*, *5*, *9*, *10*), the results here show that natural forcing, despite lacking a trend, has contributed to the modulation of the 1979–2003 global cooling evolution. If the solar and volcanic aerosol forcing were entirely absent, the temperature evolution would have comprised a steady decrease driven by Anth, but this is inconsistent with the observations (Figs. 1A and 3B). The decadal-scale temperature decline that is dominated by stratospheric ozone depletion is very likely unprecedented in the historical evolution of the lower stratospheric thermal state. The juxtaposition of the 1979–2003 ozone loss, solar variation, and volcano effects in yielding the observed temperature evolution is also likely to be unusual.

In the 21st century, if ozone depletion were to continue unabated, the anthropogenic effects would outweigh natural forcing to an even greater extent than in the 1990s (Fig. 3B). The comparable value of the Nat cooling anomaly to that of Wmgg in years without volcanic

aerosol influences (Fig. 3B) presents another implication for the future. With stratospheric ozone anticipated to recover over the next several decades because of a reduction in halogen loadings, and before the continued increase in well-mixed greenhouse gases enables them to be the dominant factor, natural forcing contributions to the global lower stratospheric temperature evolution could become relatively more important than in the period considered here.

An uncertainty in these quantitative estimates is the incomplete knowledge of the global vertical profile of ozone change immediately after volcanic eruptions (*25*, *26*). Enhancements in global ozone loss due to volcanic aerosols, as indicated by chemical modeling (*27*) and not incorporated in the data set used here, could add to the simulated cooling. Including explicit stratospheric chemistry (*28*, *29*) in future coupled atmosphere-ocean climate model simulations would provide further insights into the evolution of cooling. An increase in lower stratospheric water vapor will also contribute to a cooling (*8*, *30*), although its space-time evolution is uncertain (*31*). Another uncertainty is the extent to which upper tropospheric species (such as aerosols) affect T4 temperatures. The observed variations in stratospheric ozone likely contain influences due to dynamical fluctuations, such as changes in planetary waves and stratosphere-troposphere coupling (*1*) and the perturbation of tropical stratospheric circulation due to volcanic aerosol heating (*32*). This suggests that internal system variations in ozone will affect global temperature changes, but probably much less than the large anthropogenic ozone loss over the period. Despite the uncertainties, the simulations described here quantitatively demonstrate the existence of an externally forced response in the observed 1979–2003 global lower stratospheric temperature time series, and they delineate the natural and anthropogenic influences on the evolution of the cooling.

References and Notes

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17. Zonally and monthly averaged concentrations of stratospheric ozone are based directly on observations involving a combination of satellite and polar ozonesonde measurements [(*25*, *26*) see SOM]. A standard regression analysis of the data over the period is performed, comprising terms that represent solar variations and QBO influences, along with a function representing the stratospheric ozone loss trend based on the time series of the equivalent effective stratospheric chlorine (*26*). In view of the dominant influence of the halogen-induced depletion in the lower stratosphere over the period, stratospheric ozone change is taken to be part of the anthropogenic forcing.
18. Spatial distribution and temporal variations of tropospheric short-lived species (ozone and aerosols) are simulated by a global chemistry-transport model [(*34*, *35*) see SOM]. The vertical profiles of tropospheric and stratospheric ozone distributions are smoothly merged at the tropopause. Tropospheric aerosol species consist of dust, sea salt, black and organic carbon, and sulfate, with the respective optical properties taken from (*36*); dust and sea salt are presumed to be unaffected by anthropogenic activity.
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Supporting Online Material

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Molecular Linkage Between the Kinase ATM and NF-κB Signaling in Response to Genotoxic Stimuli

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The transcription factor NF-κB modulates apoptotic responses induced by genotoxic stress. We show that NF-κB essential modulator (NEMO), the regulatory subunit of IκB kinase (IKK) (which phosphorylates the NF-κB inhibitor IκB), associates with activated ataxia telangiectasia mutated (ATM) after the induction of DNA double-strand breaks. ATM phosphorylates serine-85 of NEMO to promote its ubiquitin-dependent nuclear export. ATM is also exported in a NEMO-dependent manner to the cytoplasm, where it associates with and causes the activation of IKK in a manner dependent on another IKK regulator, a protein rich in glutamate, leucine, lysine, and serine (ELKS). Thus, regulated nuclear shuttling of NEMO links two signaling kinases, ATM and IKK, to activate NF-κB by genotoxic signals.

The NF-κB family of transcription factors is an important point of convergence for many signal transduction pathways, which regulate genes that are critical for processes such as development, innate and adaptive immune responses, cell migration, and apoptosis (*1*). NF-κB regulates apoptosis and is an attractive target for anticancer drug development (*2*). Inactive NF-κB is cytoplasmically localized because of its association with inhibitor proteins, such as IκBα. Distinct signaling

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cascades induced by stimuli such as tumor necrosis factor α (TNFα), bacterial lipopolysaccharide (LPS), and genotoxic agents elicit NF-κB–dependent transcription by activating the cytoplasmic IKK complex—composed of two catalytic subunits, IKKα (also called IKK1) and IKKβ (also called IKK2)—and a regulatory subunit, NEMO (also called IKKγ). Another important regulatory component of the IKK complex is a protein called ELKS (*3*). Upon activation, IKK phosphorylates IκB to promote its ubiquitin-dependent degradation. Liberated NF-κB then migrates into the nucleus and induces transcription of target genes.

ATM is a nuclear protein kinase that regulates apoptosis and cell cycle checkpoint responses after DNA double-strand breaks (DSBs) (*4*, *5*). ATM is crucial for NF-κB activation by multiple DNA-damaging anticancer agents, including ionizing radiation (IR), the topoisomerase I inhibitor camptothecin (CPT), and the topoisomerase II inhibitors etoposide (VP16) and adriamycin/doxorubicin (*6*–*9*). NF-κB activation by CPT and VP16, but not by TNFα or LPS, requires a modification of free NEMO by small ubiquitin-like modifier 1 (SUMO-1) to promote its nuclear localization, which is then followed by its ubiquitination and activation of cytoplasmic IKK (*6*). Because ATM appeared critical for the latter modification of NEMO, we considered the possibility that ATM regulates NEMO activity by direct phosphorylation. ATM specifically phosphorylates serine or threonine residues followed by glutamine (Ser-Gln or Thr-Gln sites) (*10*). We identified five Ser-Gln motifs—Ser^{8}, Ser^{85}, Ser^{156}, Ser^{364}, and Ser^{383}—that are candidate phosphorylation sites for ATM in the human NEMO sequence (fig. S1A).

To determine whether any of these Ser-Gln sites are required for NF-κB activation by DNA-damaging agents in vivo, we stably introduced Myc-tagged NEMO mutants with each of these serine residues substituted with alanine into the NEMO-deficient murine pre–B cell line 1.3E2 (fig. S1, B and C). NF-κB activation by multiple DSB-inducing agents or by LPS was defective in 1.3E2 cells but was restored by stable expression of wild-type NEMO (Fig. 1, A to C). NEMO with Ser^{85}→Ala (NEMO-S85A) was the only mutant that completely failed to permit NF-κB activation by VP16 and CPT, whereas it fully restored activation by LPS (Fig. 1, B and C, and fig. S1C). Western blot analysis of IκBα indicated that this defect was associated with the loss of IκBα degradation in response to these agents (Fig. 1B), a finding consistent with the lack of DSB-inducible activation of IKK (Fig. 1D). The NF-κB activation defect in NEMO-S85A cells correlated with the loss of VP16-dependent transcription of the IκBα gene, a well-established NF-κB transcriptional target (Fig. 1E). Additionally, substitution of Gln^{86} to either alanine or asparagine caused

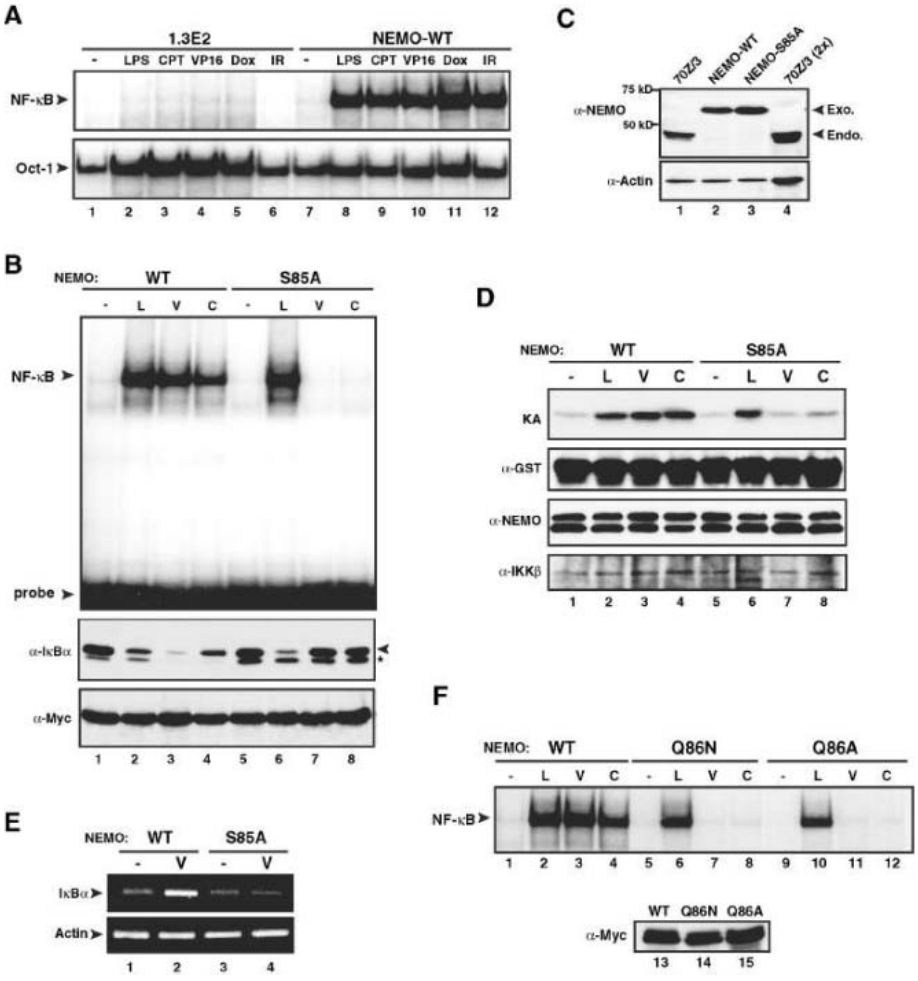

Fig. 1. Requirement of Ser^{85} of NEMO for NF-κB activation by DSB inducers. (**A**) NF-κB activation in NEMO-deficient 1.3E2 cells, or in those reconstituted with NEMO-WT, treated and analyzed by electrophoretic mobility shift assay (EMSA) (*23*). Dox, doxorubicin; Oct-1, octamer binding protein 1. (**B**) NF-κB activation and IκBα degradation in NEMO-WT and NEMO-S85A cells treated with LPS (L), VP16 (V), or CPT (C) were analyzed by EMSA and by anti-IκBα and anti-Myc Western blotting. The arrowhead points to IκBα protein, and the star indicates a possible IκBα degradation product. (**C**) Amounts of NEMO and actin from whole-cell lysates of 70Z/3, NEMO-WT, and NEMO-S85A cells were analyzed by Western blotting with antibodies (α) to NEMO and actin. Protein loading amount in lane 4 is two times the protein in lane 1. Exo., exogenous; Endo., endogenous. (**D**) IKK activation in NEMO-WT and NEMO-S85A cells treated with indicated agents were analyzed by immune complex kinase assay (*23*). KA, kinase assay. (**E**) IκBα mRNA levels in HEK293 cells as measured by reverse transcription polymerase chain reaction analysis. (**F**) NF-κB activation and Myc-tagged NEMO protein in NEMO with Gln^{86}→Asn (Q86N) and NEMO with Gln^{86}→Ala (Q86A) cells as analyzed by EMSA and Western blottting.

NF-κB activation defects in response to DNA damaging agents that were indistinguishable from those of the NEMO-S85A mutant (Fig. 1F). Thus, Ser^{85} and Gln^{86} of NEMO are each required for DSB-inducible NF-κB activation but have no apparent role in the LPS signaling pathway.

To determine whether ATM could phosphorylate NEMO on Ser^{85}, we generated glutathione-*S*-transferase (GST)–fusion NEMO wild type (WT), NEMO-5A (in which all five serines are mutated to alanine), and NEMO-S85A recombinant proteins and used them in an in vitro ATM immunocomplex-kinase assay (*11*).

Ser^{85}-dependent phosphorylation of NEMO was evident (fig. S2A). We also generated rabbit polyclonal antibodies that specifically recognized the phosphorylated Ser^{85} residue of NEMO (anti-pS85-NEMO). Anti-pS85-NEMO detected VP16-inducible and phosphatase-sensitive modification of NEMO-WT, but not of NEMO-85A, expressed in human embryonic kidney (HEK) 293 cells (Fig. 2A). CPT, IR, and doxorubicin also induced Ser^{85} phosphorylation of NEMO, but TNFα did not (Fig. 2B). Endogenous NEMO underwent inducible Ser^{85} phosphorylation, which was prevented by ATM-specific RNA interfer-

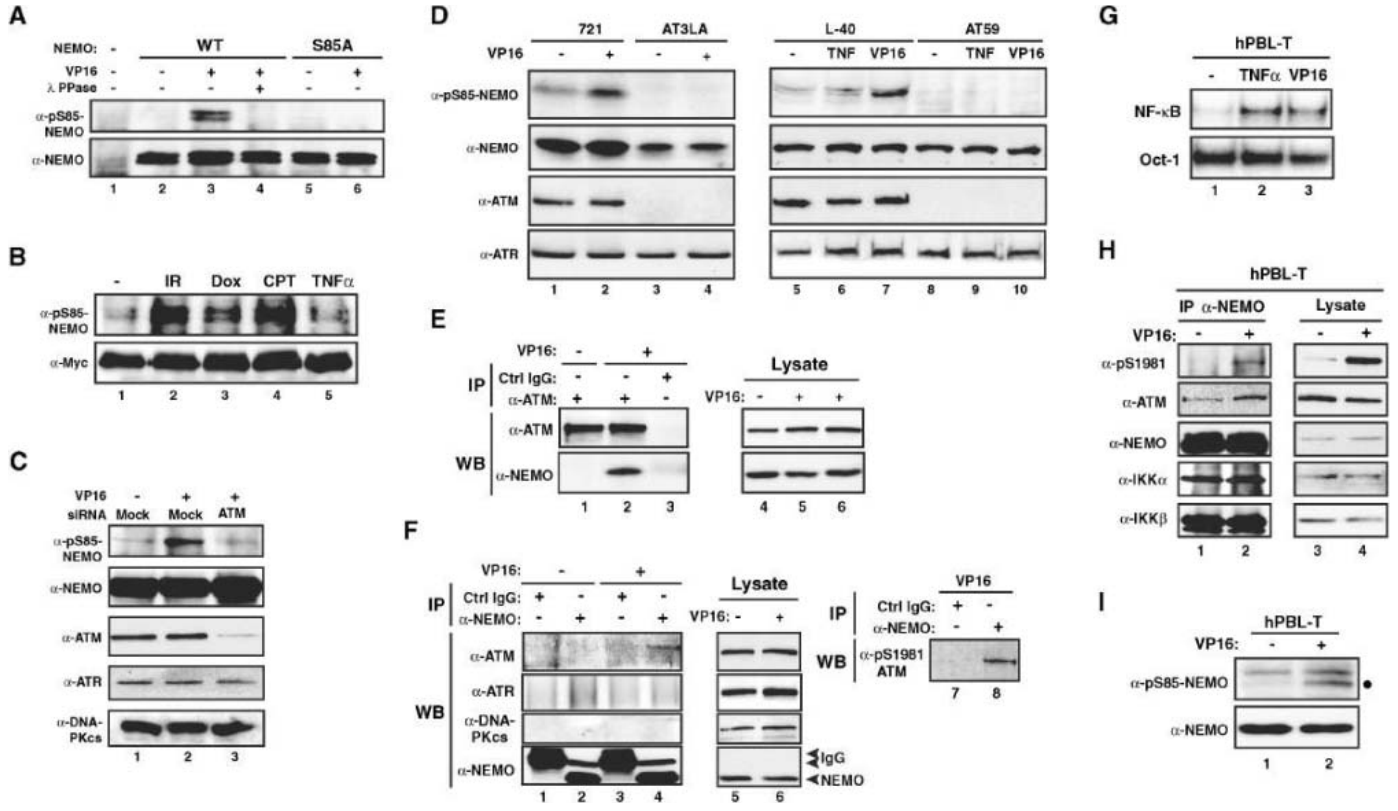

Fig. 2. Phosphorylation on Ser^{85} of NEMO by ATM. (**A**) Phosphorylation of NEMO-WT or NEMO-S85A transfected in HEK293 cells after VP16 treatment. NEMO proteins were immunoprecipitated (IP) with an antibody to Myc then analyzed by Western blotting (WB) with antibodies (α) to phospho-S85 (pS85)–NEMO and NEMO. The sample in lane 4 was treated with λ-phosphatase (λ-PPase) for 30 min after immunoprecipitation. (**B**) Induction of Ser^{85} phosphorylation of NEMO in HEK293 cells stably expressing NEMO-WT in response to CPT, IR, and doxorubicin (Dox), but not TNFα. (**C**) ATM siRNA prevents phospho-S85-NEMO detection in HEK293 cells. Whole-cell extracts were analyzed with antibodies to ATM, ATR, and DNA-PKcs. (**D**) Ser^{85} phosphorylation of NEMO is detectable in AT WT cells (721 and L40) but not in AT lymphoblast cells (AT3LA and AT59). (**E**) Coimmunoprecipitation of endogenous NEMO with an antibody to ATM in HEK293 cells. (**F**) Coimmunoprecipitation of endogenous ATM, but not ATR and DNA-PKcs, with an antibody to NEMO in HEK293 cells. NEMO-associated ATM is reactive to the antibody to phospho-S1981 ATM. (**G**) NF-κB activation in normal hPBL-T cells treated with TNFα or VP16 as analyzed by EMSA. (**H**) Coimmunoprecipitation of endogenous ATM, IKKα, and IKKβ with an antibody to NEMO in normal hPBL-T cells. (**I**) Induction of Ser^{85} phosphorylation of NEMO in normal hPBL-T cells. Black circle to the right indicates phosphorylated NEMO.

ence (RNAi) (Fig. 2C). Moreover, AT cell lines (AT3LA and AT59) failed to induce NEMO Ser^{85} phosphorylation upon exposure to VP16 (Fig. 2D). Mutation of Ser^{85} blocked VP16-inducible incorporation of ortho-^{32}P into NEMO in vivo (fig. S2B), suggesting that Ser^{85} is the predominant site of DSB-inducible phosphorylation.

Consistent with the notion that ATM is a direct NEMO kinase, endogenous NEMO and ATM could be coimmunoprecipitated in a VP16-inducible manner (Fig. 2, E and F). NEMO-associated ATM interacted with a phospho-Ser^{1981} antibody (pS1981) that detects activated ATM (*12*). VP16-inducible interaction of NEMO was not detected with other ATM family members, DNA-dependent protein kinase catalytic subunit (PKcs), or ATM-Rad3-related (ATR) (Fig. 2F). Thus, ATM appears to selectively associate with NEMO to promote NF-κB activation under these conditions. The interaction of ATM and NEMO was also detected in normal human peripheral blood (hPBL)–T cells, which correlated with Ser^{85} phosphorylation of NEMO and NF-κB activation in these normal human cells (Fig. 2, G to I).

NEMO undergoes sequential SUMO-1 and ubiquitin modifications in response to treatment of cells with VP16 (*6*). Ubiquitination (Fig. 3A) but not SUMOylation (fig. S3A) of NEMO was decreased in cells expressing the NEMO-S85A mutant. Because the migration of ubiquitinated NEMO during gel electrophoresis was consistent with mono-ubiquitination, we hypothesized that phosphorylation of NEMO on Ser^{85} was required for its mono-ubiquitination. Therefore, we tested whether direct fusion of a ubiquitin moiety to NEMO-S85A could bypass the requirement of Ser^{85} phosphorylation for activation of IKK and NF-κB, a test that has been done previously to study transactivation domain function (*13*). To this end, we generated 1.3E2 cells stably expressing NEMO-S85A with an N-terminal ubiquitin tag (Ub-S85A) that lacked the C-terminal diglycine residues of ubiquitin and was joined to the first methionine of NEMO (fig. S3B). This modified NEMO did not appear to be rapidly degraded by the ubiquitin-fusion degradation pathway (*14*). Ub-S85A NEMO supported activation of IKK (Fig. 3C) and NF-κB (Fig. 3B) in response to VP16 and CPT treatments, whereas NEMO-S85A with a similarly fused N-terminal SUMO-1 tag did not (fig. S3, B and C). Ubiquitin fusion also reversed the NF-κB activation defects of NEMO-S85A in response to both IR and doxorubicin (Fig. 3, D and E). Furthermore, clonogenic survival assays demonstrated that NEMO-S85A cells were more radiation sensitive than were NEMO-WT cells, and this effect of the NEMO-S85A mutant was also reversed by ubiquitin fusion (Fig. 3F). Thus, ATM-dependent phosphorylation and ubiquitination of NEMO was also essential for a NF-κB–dependent cell survival response.

Mono-ubiquitination can regulate protein targeting, such as endocytic trafficking and sorting (*15*), and was also implicated in nuclear export (*16*). We thus tested whether NEMO-S85A had a nuclear export defect that could be reversed by ubiquitin fusion. Nuclear immunostaining of NEMO-WT increased up to 120 min

after VP16 treatment of cells immobilized on a glass chamber and then declined by 180 min (Fig. 4A and fig. S4, A to D). NF-κB activation, as detected by nuclear staining of p65, peaked at 180 min under these conditions (fig. S4, C and D). The peak levels of NEMO and p65 nuclear staining varied from ~20 to 40% of exposed cells in different experiments, which correlated with the overall magnitude of NF-κB–dependent survival that we observed in this cell system (Fig. 3F). Nuclear staining of NEMO-S85A after VP16 treatment occurred with comparable kinetics to those of NEMO-WT but remained at the peak levels even 240 min after stimulation (Fig. 4A). This nuclear export defect of NEMO-S85A was bypassed by ubiquitin fusion (Fig. 4A).

Polyubiquitination of certain proteins in the NF-κB signaling pathway, including NEMO, is proposed to provide a scaffold to assemble IKK signaling complexes to promote activation of IKK and NF-κB by immune and inflammatory stimuli (*17*). To probe the potential role of ubiquitin in the assembly of an IKK signaling complex in the DSB-dependent signaling pathway, we screened ATM and NEMO immunoprecipitates for the presence of other signaling proteins by immunoblotting. Anti-ATM precipitates prepared from NEMO-WT cells, but not those prepared from NEMO-S85A cells, contained IKKβ in a VP16-inducible manner (Fig. 4B). ATM was also detected in anti-IKKβ immunoprecipitates (Fig. 4C). Because IKKβ remains cytosolic in the NF-κB activation pathway induced by genotoxic stress (*6*), these results led us to test whether a fraction of ATM exits the nucleus upon DNA damage. Subcellular fractionation studies demonstrated that a small fraction of activated ATM was exported from the nucleus in a NEMO-dependent manner (Fig. 4, D and E). ATM immunoprecipitates prepared from cytoplasmic extracts demonstrated VP16-inducible presence of IKK activity (Fig. 4F). We used control immunoglobulin G (IgG) and a mutant GST-IκBα (Ser-Ser/Ala-Ala) substrate containing alanine substitutions at both IKK sites to demonstrate the specificity of IKK activity associated with cytoplasmic ATM. The relatively low amounts of IKK activity derived from ATM immunoprecipitates may be due to inefficiency of immunoprecipitation with antibodies to ATM. However, IKK activity was absent in anti-ATM immunoprecipitates prepared from NEMO-S85A cells. Moreover, this defect of NEMO-S85A to promote ATM-IKK interaction was reversed by ubiquitin fusion (Fig. 4G). Thus, the nuclear export function of NEMO was necessary to promote the ATM-IKK association and IKK activation in the cytoplasm. Moreover, NF-κB activation in Ub-S85A cells was completely blocked by the ATM inhibitors wortmannin and caffeine (fig. S4E). These findings indicate that ATM has another function in the cytoplasm: mediating the activation of IKK and NF-κB.

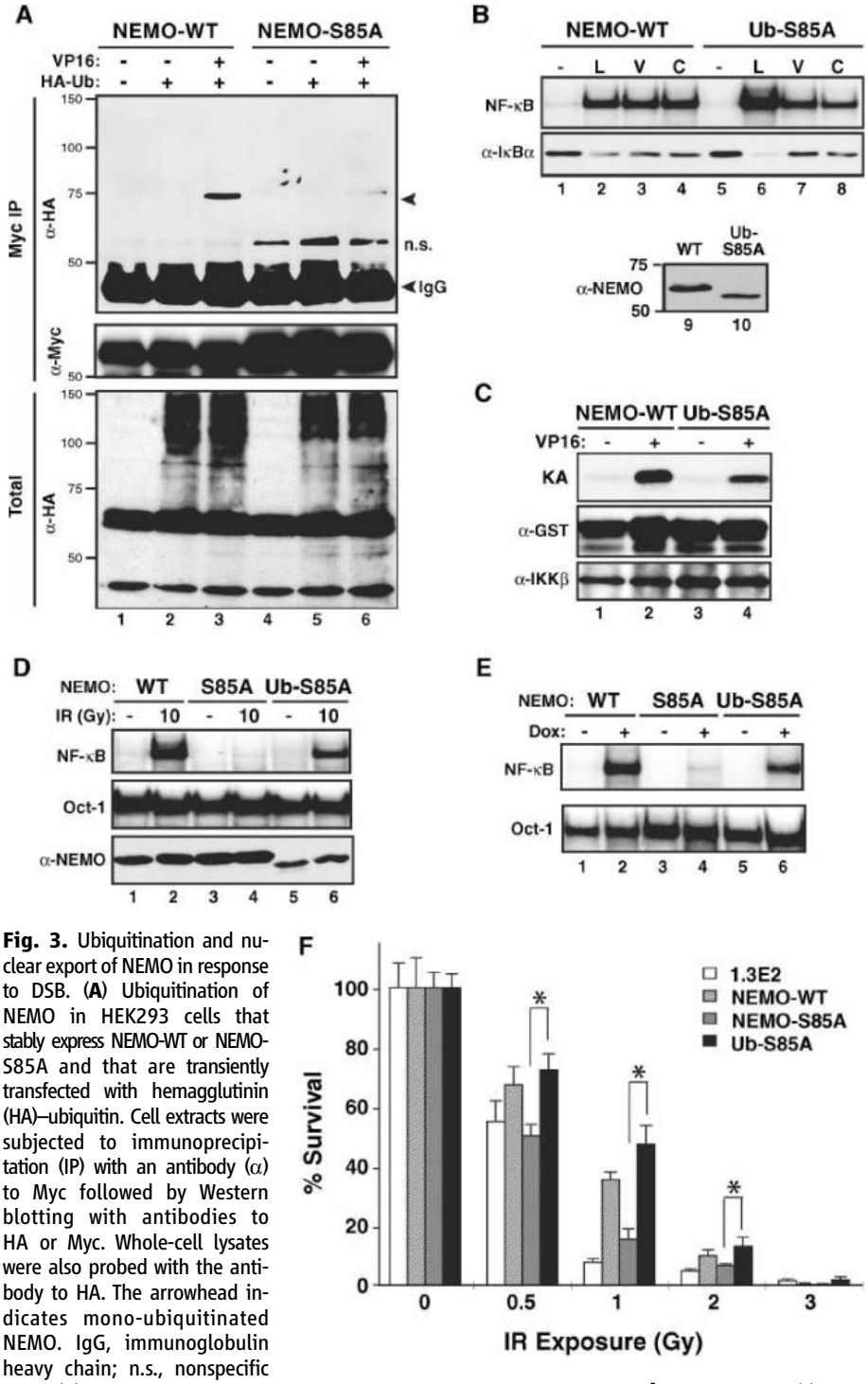

Fig. 3. Ubiquitination and nuclear export of NEMO in response to DSB. (**A**) Ubiquitination of NEMO in HEK293 cells that stably express NEMO-WT or NEMO-S85A and that are transiently transfected with hemagglutinin (HA)–ubiquitin. Cell extracts were subjected to immunoprecipitation (IP) with an antibody (α) to Myc followed by Western blotting with antibodies to HA or Myc. Whole-cell lysates were also probed with the antibody to HA. The arrowhead indicates mono-ubiquitinated NEMO. IgG, immunoglobulin heavy chain; n.s., nonspecific band. (**B**) NF-κB activation and NEMO levels in NEMO-WT and Ub-S85A cells [treated with LPS (L), VP16 (V), or CPT (C)] as analyzed by EMSA and Western blotting, respectively. (**C**) IKK activation in NEMO-WT and Ub-S85A cells, as analyzed in Fig. 1D. (**D** and **E**) NF-κB activation in NEMO-WT, NEMO-S85A, and Ub-S85A cells after (D) IR treatment and (E) doxorubicin (Dox) treatment. Gy, grays. (**F**) Increased sensitivity to radiation of cells lacking NEMO is reversed by NEMO-WT and Ub-S85A but not by NEMO-S85A. 1.3E2, NEMO-WT, NEMO-S85A, and Ub-S85A cells were exposed to indicated doses of IR and analyzed for clonogenic survival assay (*23*). The average + SD of three experiments is plotted for each condition. Asterisks indicate that $P < 0.05$ in both an ANOVA and a Tukey's test.

Previous studies implicated several cytoplasmic proteins in NF-κB activation after genotoxic stress, including receptor interacting protein 1 (RIP1) (*18*) and p90 ribosome S6 kinase (RSK1) (*19*). Reduction of RIP1 and RSK1 protein levels by RNAi had a small inhibitory effect on NF-κB activation by VP16. In contrast, RNAi reduction of ELKS, a newly discovered

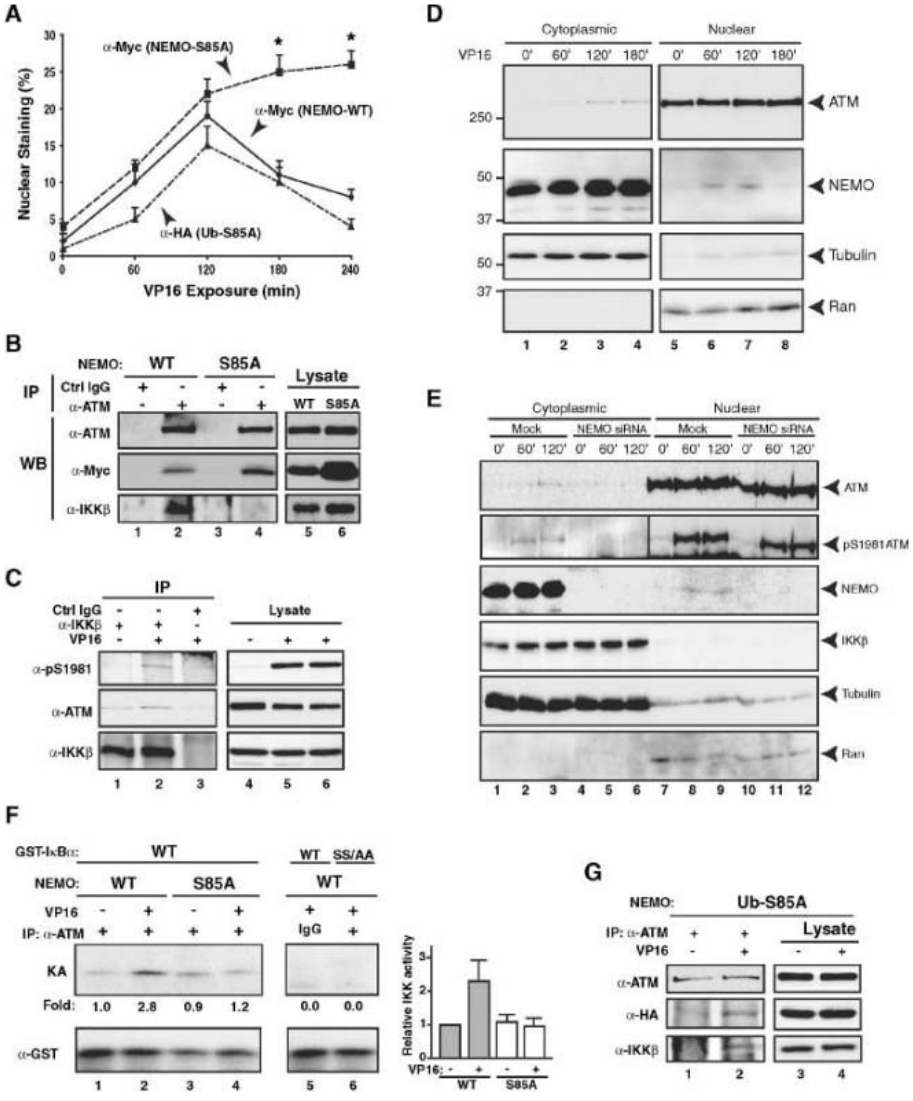

Fig. 4. Mono-ubiquitination of NEMO promotes assembly of an ATM:IKK complex in the cytoplasm. (**A**) A graph representing the percentage of cells showing nuclear staining of NEMO in NEMO-WT, NEMO-S85A, and Ub-S85A cells treated with VP16 for indicated times and measured by immunofluorescence with the use of an antibody (α) to Myc (*23*). The average + SD of triplicate studies is plotted for each condition. Asterisks indicate that $P < 0.05$ in both an ANOVA and a Tukey's test. (**B**) Coimmunoprecipitation of endogenous IKKβ with an antibody to ATM in NEMO-WT, but not in NEMO-S85A cells, after VP16 exposure. IP, immunoprecipitation; WB, Western blotting. (**C**) Coimmunoprecipitation of endogenous activated ATM with an antibody to IKKβ in HEK293 cells. (**D**) Detection of nuclear NEMO and ATM in the cytoplasm of HEK293 activated cells after VP16 exposure for indicated times. (**E**) NEMO siRNA prevents the appearance of cytoplasmic activated ATM in HEK293 cells. In the pS1981ATM panel, lanes 1 to 6 were exposed longer than lanes 7 to 12 by three times to reveal the lack of activated ATM in lanes 4 to 6 even after a long exposure. (**F**) IKK activity present in cytoplasmic ATM complexes in NEMO-WT and NEMO-S85A cells treated with VP16. Average ± SD is shown for each condition ($n = 3$). (**G**) Coimmunoprecipitation of endogenous ATM with Ub-S85A after VP16 exposure.

component of the cytoplasmic IKK complex (*3*), caused a severe NF-κB activation defect similar to that of NEMO reduction (Fig. 5A). The inhibitory effect of ELKS RNAi on NF-κB activation could not be attributed to a defect in Ser^{85} phosphorylation of NEMO or ATM-IKK interaction (Fig. 5, B and C). IKK activation was, however, strongly inhibited by ELKS RNAi (Fig. 5D). Interestingly, ATM associated with ELKS in a VP16-dependent manner (Fig. 5E). These findings suggest that ELKS is a downstream regulator that is essential for ATM-dependent IKK activation in response to DSBs.

Our results collectively demonstrate that ATM phosphorylates Ser^{85} of NEMO in response to genotoxic stress and that this event is required for mono-ubiquitination of NEMO. This modification is essential for nuclear export of NEMO and ATM and for their subsequent interaction with the catalytic IKK subunit in the cytoplasm. Although potential cytosolic function for ATM in the regulation of β-adaptin and phosphorylation of the eukaryotic initiation factor 4E–binding protein 1 have been previously reported (*20*, *21*), we demonstrate a previously unrecognized mechanism for stimulus-dependent nuclear export of ATM. In addition, our data demonstrate that ELKS is a critical component of DNA damage-induced IKK activation, acting downstream of cytosolic ATM-IKK complex formation. Because ATM also associates with ELKS upon genotoxic stress induction, we propose a model in which a cytosolic signaling complex containing NEMO, ATM, IKK catalytic subunits, and ELKS is assembled in response to genotoxic stress to mediate NF-κB activation (Fig. 5F). This model contrasts with those proposed for NF-κB activation in response to immune and inflammatory signals where Lys^{63}-linked polyubiquitination plays a critical role in IKK activation (*22*). Despite the differences in the mechanisms and types of ubiquitination involved, our model also suggests that the role of ubiquitin in assembling an IKK signaling complex is a conserved strategy that has evolved to regulate NF-κB in response to DNA damage and immune and inflammatory signals.

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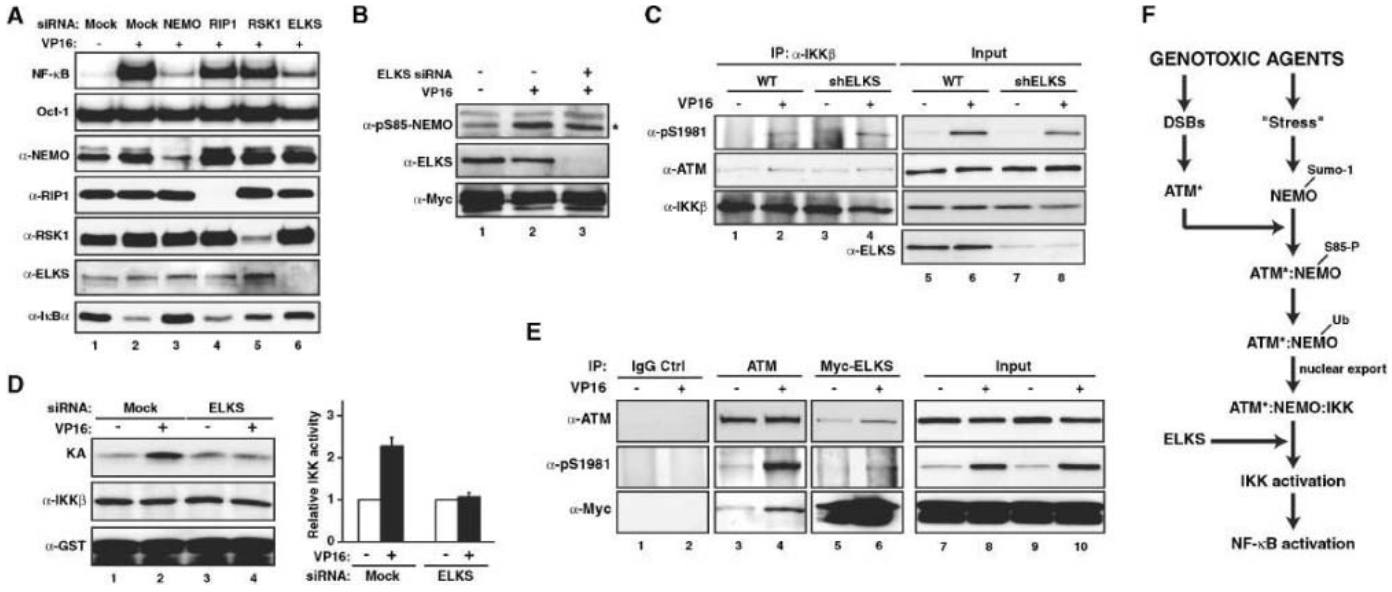

Fig. 5. Requirement of ELKS for IKK activation downstream of ATM:IKK complex formation. (**A**) Activation of NF-κB in response to VP16 in the presence of siRNA against NEMO, ELKS, RIP1, or p90RSK1. Western blot analyses of corresponding proteins are also shown. (**B**) Effects of ELKS depletion on Ser^{85} phosphorylation of NEMO. Asterisk indicates NEMO. (**C**) Effects of ELKS depletion on interaction of ATM and IKK. shELKS, small hairpin ELKS stable cell clone. (**D**) Effects of ELKS depletion on IKK activation. Average ± SD for each condition is shown (n = 3). (**E**) Association of ATM with ELKS. HEK293 cells were transfected with a Myc-ELKS-His construct and either left untreated or treated with VP16. Whole-cell lysates were immunoprecipitated (IP) with control IgG or antibodies (α) to ATM or Myc and analyzed by Western blotting with antibodies as indicated. (**F**) A model depicting the role of NEMO modifications, ATM-dependent events, and the point at which ELKS regulates activation of IKK and NF-κB in response to DSB inducers. Asterisks indicate activated ATM.

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Cell Type Regulates Selective Segregation of Mouse Chromosome 7 DNA Strands in Mitosis

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After chromosome replication, sister chromatid copies are generally thought to segregate randomly to daughter cells. However, sister chromatids differ in their DNA strands, with each chromatid inheriting one older strand that is paired to a newly synthesized strand. Genetic analysis with a homologous chromosome pair indicated nonrandom chromatid distribution in embryonic stem cells. Biased segregation pattern was also found in all 100 endoderm cells examined, but not in any of the 165 neuroectoderm cells. In contrast, the mesoderm, cardiomyocyte, and pancreatic cells exhibited a random mode of segregation. Strand distribution mechanisms regulated by cell type may have consequences for cellular differentiation and for evolving strategies for developmental mechanisms.

Each chromosome replication produces two paired daughter chromosomes, called chromatids. The WC′ chromatid contains the "older" "Watson" strand as it is derived from the parental chromosome and the newer, complementary "Crick" (C′; ′ indicates newer strand) strand, so the sister W′C chromatid contains a newer W′ and an older C strand (Fig. 1). One chromatid from each chromosome is delivered to each daughter cell during mitosis. The chromatids from a homologous pair of chromosomes are distributed randomly to daughter cells, where they are again referred to as chromosomes. A recent embryonic stem (ES) cell chromosome (Chr 7) recombinants study (*1*) was said to indicate selective sorting of DNA strands in mitosis (*2*). Cairns' theory for segregating "oldest" DNA strands of all the chromosomes to asymmetrically self-renewing "stem" cells was proposed previously as a device to protect cells from inheriting DNA replication errors so as to avoid future cancer development (*3*). Another theory for cellular differentiation exploiting the sequence differences between DNA strands (*4*), the somatic strand-specific imprinting and patterned segregation (SSIS) model, was said to produce nonequivalent daughter cells in mitosis for development (*2*, *5*). The model postulated a selective segregation mechanism in which one daughter cell inherits WC′+WC′ and the other inherits W′C+W′C chromatids from homologs of a specific chromosome. That is, by specifying parental "older" chromosome strands to simplify their presentation, the term WW:CC (Fig. 1) was coined to indicate a nonrandom strand-chromatid segregation pattern (*2*).

We asked here whether the chromosome-specific nonrandom strand segregation process exists in biology. We are not aware of any study designed specifically to determine the strand

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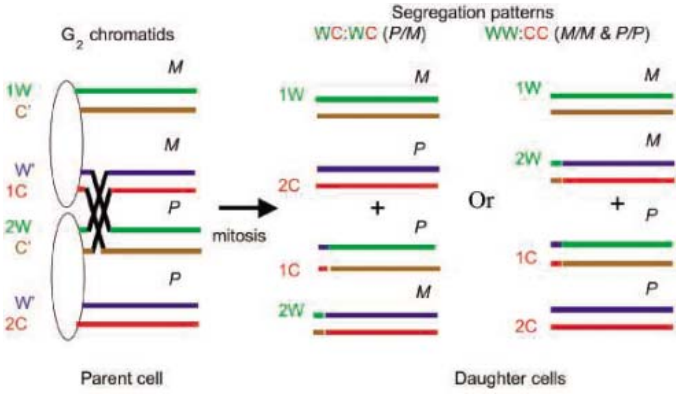

Fig. 1. Diagrams of Chr 7 strand segregation patterns following mitotic site-specific recombination [modified from (*2*)]. The numbers indicate "older" strands and resulting chromatids derived from maternal (no. 1) and paternal (no. 2) parental chromosomes, respectively. Only the specifically designated W or C parental chromosomes strands are indicated in daughter cells to simplify their representation. The WC:WC pattern in the progenitor cell is indicated when *HAT*^{r} *M/P* recombinant is obtained (fig. S1), and the WW:CC pattern is reflected by the *HAT*^{r} *M/M* recombinant. Note that only WC′ and W′C chromatids must have recombined in G_2 phase in the examples diagrammed here. Alternatively, should recombination be restricted to both WC′ or both W′C chromatids, the *M/P HAT*^{r} recombinants will result from the WW:CC pattern and *M/M HAT*^{r} ones from the WC:WC pattern (not diagrammed). To help follow strand distribution, the parental W is colored green, parental C in red, and the newest strands are represented by brown (C′) and blue (W′) colors. The crossover is indicated as X; *P* is paternal *Snrpn* epiallele, *M*, maternal epiallele; and ellipses represent centromeres.

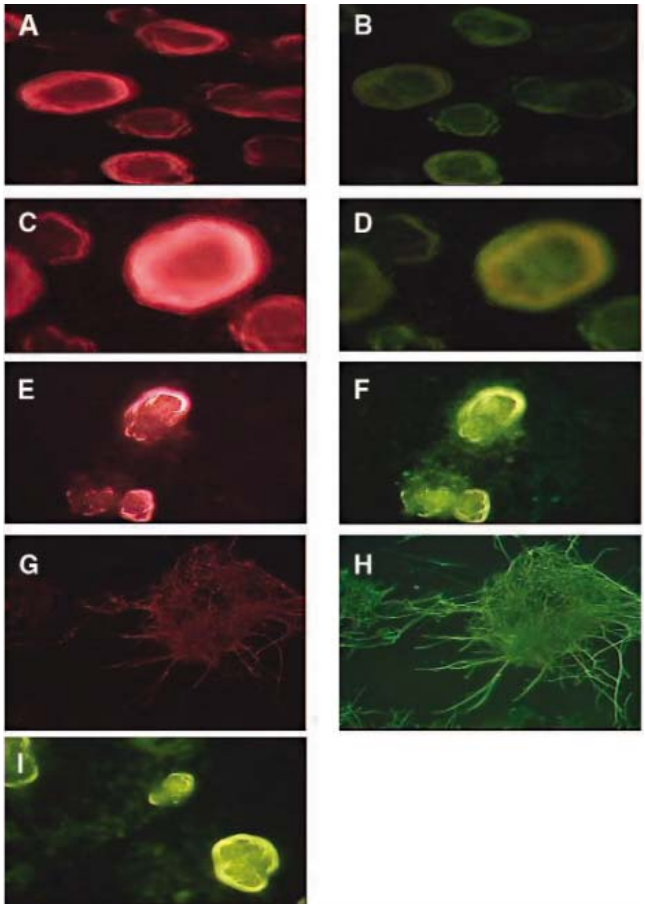

Fig. 2. Detection of cell differentiation protein expression markers by immunofluorescence microscopy. (**A**) FOXA2 and (**B**) SOX17 of endoderm cells; (**C**) glucagon and (**D**) nestin of insulin-secreting pancreatic cells; (**E**) desmin and (**F**) smooth muscle actin of cardiomyocytes; (**G**) Tuj-1 and (**H**) nestin of neuroectoderm cells; (**I**) FEC6 of early mesodermal cells.

distribution of a specific chromosome. We fortuitously noted an unusual result in a recent study in which all 432 Chr 7 mitotic recombinants had become homozygous for a marker located distal to the crossover point in mouse ES cells. It was postulated that the *Loxp-Cre*–induced mitotic recombination system that was used might have somehow nonrandomly placed recombinant chromatids at the metaphase plate, causing them to segregate away from each other and resulting in homozygosis (*1*). An alternative model (*2*) advanced was that ES cells might inherently follow a nonrandom pattern, such as the WW:CC pattern diagrammed in Fig. 1. It was further speculated in the SSIS model that mechanisms for alternative segregation patterns may have evolved and that different sets of chromosomes might follow specific distribution patterns in different cell types. We asked here whether the cell type regulates the Chr 7 segregation pattern in mouse cells. Indeed, it does.

We used the model (*1*) consisting of recombination cassettes introduced near the Chr 7 centromeres in DT1E9 cells (fig. S1). Both cassettes contain complementary but nonfunctional halves of the human hypoxanthine phosphoribosyl transferase (*HPRT1*) minigene, with a *Loxp* recombination site embedded in the *HPRT1* gene's second intron. Interchromosomal recombination between *Loxp* sites in each cassette produced an intact *HPRT1* minigene that was scored by the ability of *Hprt*-deficient ES cells to grow in HAT (hypoxanthine, aminopterin, and thymine) medium (HAT resistance, *HAT*^{r}). The strand segregation pattern of recombinants was followed both by molecularly monitoring the *Snrpn* gene's promoter region "epialleles," located distal to the crossover point near the tip of the chromosome, by Southern analysis, and by assessing phenotype of drug markers contained in the recombination cassettes (fig. S1). The paternal (*P*) and the maternal (*M*) epialleles were distinguished by analyzing the differentially methylated parent-of-origin–specific "imprinting" state due to cytosine base methylation at a specific Sac II restriction site of the *Snrpn* gene's promoter region, as described previously (*1*).

Because limited proliferation expected of differentiated cell lines prohibited our study, we generated a simian virus 40 (SV40) containing the large T-antigen gene (Phoenix retroviral expression system); its integration into the genome is known to help proliferation of mouse cells (*6*). The transformed ES line was used to derive six differentiated cell lines by "in vitro" differentiation (materials and methods in the supporting online material). Their differentiation was diagnosed by monitoring both expression of cell type–specific protein markers with immunofluorescence microscopy (Fig. 2) and morphological changes (*7*).

We wanted to know whether cell type affects strand segregation pattern. A previous study (*1*) observed that all *HAT*^{r} recombinant cell cultures tested had become homozygous [*M/M* or *P/P*, respectively, depending on whether the cell line used contained Δ5′-cassette (fig. S1) in the maternal or the parental chromosome] for the *Snrpn* epiallele marker (Fig. 1). As a control, we first found that all 20 individual colony-derived cultures analyzed from each cell type maintained *P/M* heterozygosis (*7*). This indicated that the T-antigen gene did not interfere with the epialleles' stability during cellular proliferation. As another control for the results to be presented below and to assess how well the conclusions of the previous study (*1*) can be generalized, we examined the segregation pattern of virally infected ES recombinants. The viral integration did not affect the deduced WW:CC segregation pattern, because all 20 *HAT*^{r} recombinants

tested had become *M/M* homozygous (Fig. 3). This result confirmed the previously described pattern observed with standard ES cells (*1*).

Additionally, only the WW:CC pattern was found in all the 100 endoderm cells examined (Fig. 3). In contrast, among 100 recombinant cultures examined for each class of pancreatic, mesodermal, and cardiomyocyte cells, about one-third of them were of the *P/M* type (Fig. 3; table S1). Moreover, the neuroectoderm recombinants showed yet another potentially nonrandom pattern in which all 165 cells analyzed maintained the *P/M* constitution (Fig. 3). In accord with the identification of *P* and *M* markers through Southern analysis, all *HAT*^{r} recombinant cultures exhibited the predicted puromycin and neomycin marker phenotypes (fig. S1). As the recombination frequency in cells was $<1.0 \times 10^{-3}$ (*1*) (table S1) and the *Cre* gene expression was transient (see SOM), recombination in the G_2 phase in a given cell likely involves not more than two of the four chromatids. Because of differentiation by HAT resistance, only recombination between nonsister chromatids will be selected with this procedure. These recombinants from multiple experiments were pooled for the analysis.

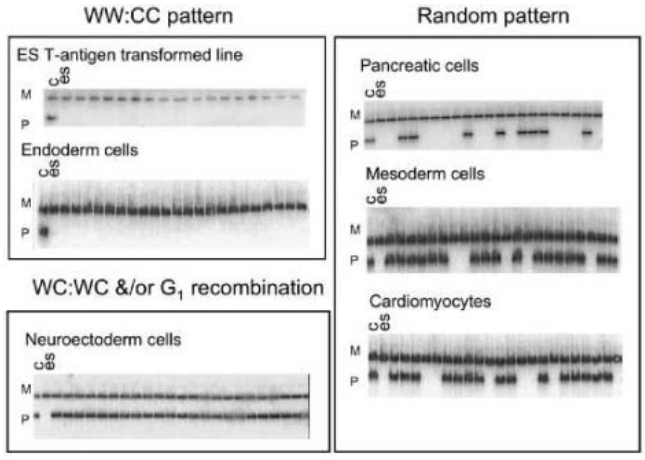

Fig. 3. Southern analysis of Chr 7 *HAT*^{r} recombinants selected from indicated cell type cultures. Southern analysis autoradiographs of Sac II plus Taq I double-digests of DNA are shown. The larger (5.3-kb) Taq I–Taq I DNA fragment indicates the maternal (*M*) *Snrpn* epiallele; the smaller (1.9-kb) Taq I–Sac II fragment reflects the paternal (*P*) form (*1*). The 850-bp Taq I–Sac II fragment was used as the radiolabeled probe. Each lane represents DNA derived from cultures grown from an individual colony. The mouse ES cells panel: Lane 1 contains DNA isolated from a culture infected with the virus containing the SV40 large T antigen; it shows the *M/P* constitution. Lane 2 contains another control, cells without viral infection and after mitotic recombination showing *M/M* homozygosis, as demonstrated previously (*1*). Lanes 3 to 20 represent cultures from independent recombinant colonies of the line that had been infected with the T-antigen gene; all the recombinants tested had become *M/M*. Results of other indicated cell types and the deduced segregation patterns are presented in other panels. The first control (C) lane in each panel represents *P/M* constitution of cells of each type. As another *M/M* control, the second lane (es) contained DNA of the virally infected ES cultures following recombination. Among nearly 100 recombinants of each cell type tested (table S1), DNA analysis of only about 20 recombinants from each cell type is displayed here.

The segregation pattern of *P/M* recombinant cultures can be explained by a combination of three possibilities. First, recombination could have occurred in the G_1 phase of the cell cycle (*8*), and such cases would be unrelated to the strand segregation issue addressed here (fig. S1). Two other nonrandom segregation possibilities after G_2 recombination have been detailed in Fig. 1. Curiously, all 165 neuroectoderm recombinant cultures were of the *P/M* type (Fig. 3). There is no reason to presume that all *P/M* recombinants in all cultures only originate from G_1 events. Moreover, for only *P/M* type to arise by G_1 recombination would require an unlikely possibility in which Chr 7 strands always follow the nonrandom pattern in all cell types. Therefore, it would not be surprising if neuroectoderm cells always followed one of the two nonrandom patterns described in Fig. 1. Thus, in principle, all hypothetical possibilities of WW:CC, WC:WC, and random patterns of chain segregation might exist in different cell types. Equally interesting possibilities are where recombination may be restricted to the G_1 or to the G_2 phase, may occur in both phases, or may occur only between two specific nonsister chromatids. Our results do not distinguish between these very interesting possibilities. We suggest that regulation of chromatid distribution to progeny cells occurs irrespective of recombination occurring between the homologs. We imagine that such recombination constraints are imposed by such a chromatid distribution mechanism itself. In this context, it is noteworthy that similar analysis of Chr 11 produced both *P/M* and *M/M* recombinants, whereas Chr 7 recombinants were only of the *M/M* type in ES cells (*1*). Thus, despite the G_1 caveat discussed above concerning Chr 11 *P/M* recombinants, different chromosome-specific segregation patterns might be predicted in cultures of the same cell type. Taken together, our data support a model where distribution of nonrandom Chr 7 strands occurs in at least some cell types, and we presume it to be random in others.

Following an explanation advanced to explain similar results with *Drosophila* (*8*, *9*), Liu *et al*. (*1*) explained the *M/M* homozygosis result discovered in ES cells by postulating a process like meiotic-reduction-division 1, in which sister chromatids remain attached and segregate together to one pole of the mitotic spindle after recombination. We believe such a model is unlikely, as it makes an unusual requirement: that chromatid segregation in mitosis occurs through chromosome regions other than the centromeres. Also, this constraint is unlikely to be imposed by two different site-specific recombination systems; the Cre system in mouse and the *FLP/FRT* system in *Drosophila* studies. Moreover, this constraint recombination in all other cell type cultures should have followed the same biased segregation pattern. However, results of the present study showed varied segregation patterns of Chr 7. In contrast, the selective strand segregation model is in accord with the centromere's usual role in segregation (Fig. 1A). From these considerations, it was proposed previously that the Chr 7 selective strand segregation process normally operates in mouse ES cells (*2*). The present study advances that notion and suggests that the pattern is not invariant, as it changes with the cell type in very interesting ways. A handful of studies concerning the entire genome investigated segregation of base-labeled strands in diverse systems and observed indications of both nonrandom (*11*, *12*) and random (*12*, *13*) distribution patterns. The Cairns "immortal strand" model (*3*) has obtained considerable support from recent studies (*14*–*19*). Rather than entire genomic strands segregating in a specific way, in addition being distinguished in age as template versus newly replicated strands, and involving asymmetric cell divisions in the Cairns model, the SSIS model instead proposes a cell type–regulated chromosome and Watson versus Crick strand-specific segregation process operating in symmetric cell divisions. Mechanisms of both models remain to be worked out. We point out that the WW:CC pattern is consistent with the SSIS model, and both WW:CC and WC:WC patterns are consistent with the Cairns model should all chromosomes follow the specific pattern during an asymmetric cell division. The SSIS model describes a mechanism for development of diploid organisms by judiciously exploiting developmentally installed epigenetic controls installed in somatic lineages for regulating developmentally important genes. We propose that the selective chromosome-specific segregation process might constitute an important mechanism for cellular differentiation and for evolving developmental mechanisms.

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Supporting Online Material

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Transient Homologous Chromosome Pairing Marks the Onset of X Inactivation

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Mammalian X inactivation turns off one female X chromosome to enact dosage compensation between XX and XY individuals. X inactivation is known to be regulated in cis by *Xite*, *Tsix*, and *Xist*, but in principle the two Xs must also be regulated in trans to ensure mutually exclusive silencing. Here, we demonstrate that interchromosomal pairing mediates this communication. Pairing occurs transiently at the onset of X inactivation and is specific to the X-inactivation center. Deleting *Xite* and *Tsix* perturbs pairing and counting/choice, whereas their autosomal insertion induces de novo X-autosome pairing. Ectopic X-autosome interactions inhibit endogenous X-X pairing and block the initiation of X-chromosome inactivation. Thus, *Tsix* and *Xite* function both in cis and in trans. We propose that *Tsix* and *Xite* regulate counting and mutually exclusive choice through X-X pairing.

The random form of X-chromosome inactivation (XCI) [reviewed in (*1*)] is regulated by a "counting" mechanism that enables XCI only when more than one X is present in a diploid nucleus. A "choice" mechanism then stochastically designates one X_a (active X), on which the X-inactivation center (*Xic*) is blocked from initiating silencing, and one X_i (inactive X), on which the *Xic* is induced to initiate chromosome-wide silencing. Regulatory elements have been mapped to three noncoding *Xic* genes, including *Xist* (*2*–*4*), its antisense partner *Tsix* (*5*–*7*), and *Xite* (*8*). Whereas *Xite* and *Tsix* together regulate counting and choice (*6*, *7*, *9*–*11*), *Xist* predominantly regulates chromosome-wide silencing (*4*, *12*–*14*). Interestingly, each gene acts in cis, with *Xite* activating the linked *Tsix* allele, *Tsix* repressing the linked *Xist* allele, and *Xist* repressing other genes on the same X.

Although cis-acting genes dominate the *Xic*, *Xic* function must extend in trans. Notably, the choice of X_a and X_i always occurs in a mutually exclusive manner, so when one X is designated X_a, the other is accordingly designated X_i. The idea of crosstalking is supported by a $Tsix^{-/-}$ knockout, in which choice becomes "chaotic" with the occurrence of 2 X_i, 1 X_i, or 0 X_i per cell (*9*, *11*). Though trans-interaction seems necessary (*9*, *15*), direct evidence has been lacking. In principle, trans-sensing could be accomplished by feedback signaling cascades, diffusible X-linked factors, or direct interchromosomal pairing such as that proposed for T cell differentiation (*16*).

Because somatic homolog pairing does not generally occur in mammals, we surmised that pairing—should it occur on the X—must take place transiently. Here, we followed the movement of the chromosomes over time using fluorescence in situ hybridization (FISH) in differentiating mouse embryonic stem (ES) cells, a model that recapitulates XCI in culture. We measured the X-X interchromosomal distances for day 0 (pre-XCI), day 2 and day 4 (XCI onset), day 6 ES cells, and mouse embryonic fibroblasts (MEFs) (Fig. 1). By combining two non-overlapping probes, we obtained 99% X detection rates (single probes gave 85 to 90% rates). Only nuclei with two resolvable signals were scored. For each experiment, 150 to 250 nuclei were scored, and similar results were obtained in three independent tests.

In wild-type XX cells, the X-X distance was highly dynamic during cell differentiation (Fig. 1A). On day 0, the interchromosomal distances approximated a normal distribution, suggesting near-randomness. Interestingly, on day 2, a high proportion of cells began to display close X-X distances, as shown by a left shift in the distribution (Fig. 1A) [Kolmogorov-Smirnov (KS) test, $P = 0.01$]. This trend continued into day 4 ($P < 0.001$) and partially returned to baseline on day 6 ($P = 0.41$). The MEF distribution was completely random, somewhat more so than for day 0 ES cells, perhaps reflecting spontaneous differentiation of some ES cells. Cumulative frequency curves (Fig. 1B) showed that day 2 and day 4 displayed the highest frequency of "promixity pairs," or pairs with normalized X-X distances (ND) <0.2 (<2.0 μ). Among proximity pairs, one-third displayed 0.2- to 0.5-μ separation (Fig. 1C), a fraction greater by factors of 6 and 16 than in day 0 ES and MEFs, respectively. X painting confirmed the presence of two Xs (fig. S1), thus excluding the possibility of visualizing sister chromatids within XO cells.

Measurement of interautosomal (A-A) distances at 1C [chromosome 1 (Chr1) centromere], *Abca2* (Chr2), and chromosome 3 centromere showed normal distributions at all time points (Fig. 1B and fig. S2), demonstrating that proximity pairing was not generally observed. To determine the extent of pairing on the X, we tested four bacterial artificial chromosome (BAC) probes in combination with an *Xic* probe (Fig. 1D and fig. S3) and found that, whereas *Xic* movement was constrained by homologous interaction, the flanking regions adopted relatively free positions, with each locus showing near-random distributions across time (fig. S3). Thus, X-X interactions were restricted to the *Xic*.

The pairing kinetics suggested linkage to XCI, which coincidentally initiates between day 2 and day 4 of differentiation. Because *Xist* RNA up-regulation is the earliest known cytologic feature of XCI (*1*), we asked whether pairing could be observed more frequently in $Xist^+$ cells. Indeed, $Xist^+$ cells showed 46% with X-X association (Fig. 2, A and B), indicating that pairing occurs just before or during *Xist* up-regulation. To pinpoint the time frame, we employed the additional temporal markers, Ezh2 and H3-3meK27, which accumulate on the X_i shortly after *Xist* up-regulation during the "early X_i maintenance" phase [reviewed in (*17*)]. On day 2, trans-associations were significantly enriched in $Ezh2^-$ cells and in $H3\text{-}3meK27^-$ cells relative to $Ezh2^+$ and $H3\text{-}3meK27^+$ cells (Fig. 2, C and D, and fig. S4). These results restricted trans-interactions to Xist-expressing cells that have not yet recruited Ezh2 and H3-3meK27, thus demonstrating a very early time frame, well before the XCI maintenance phase.

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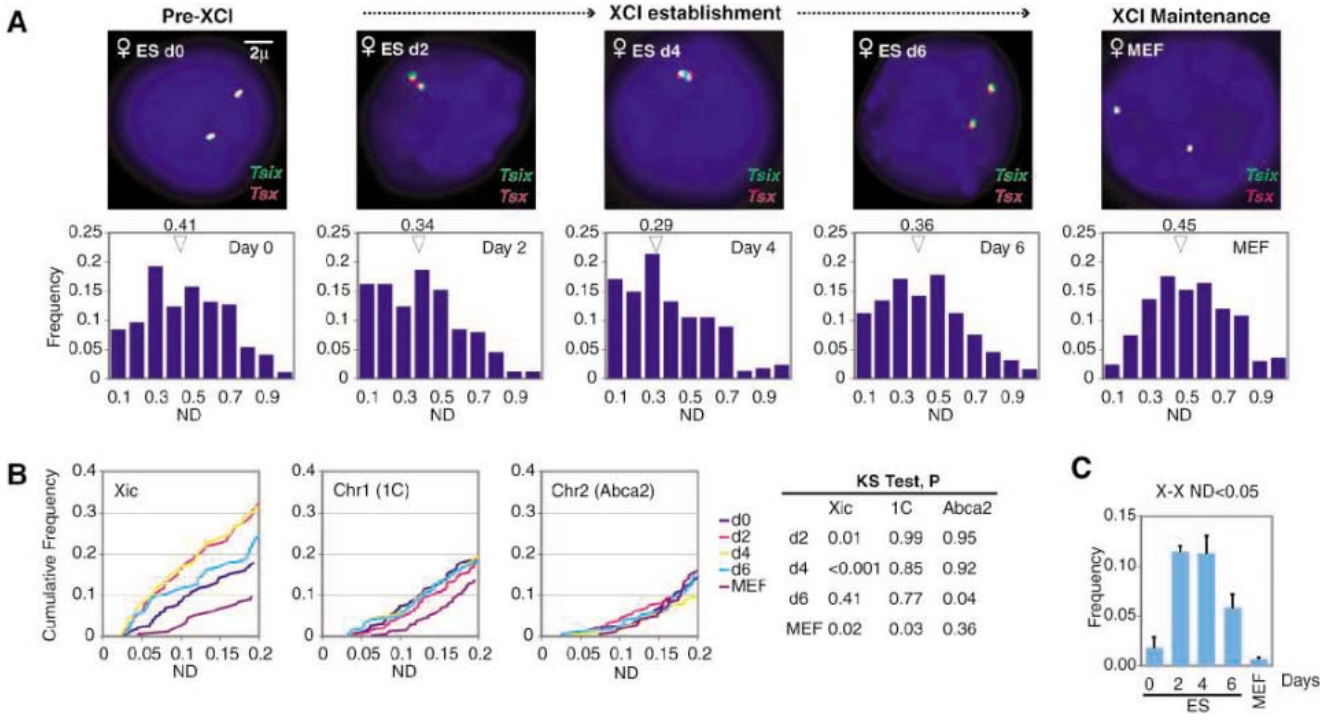

Fig. 1. Evidence for X-X homologous associations. (**A**) DNA FISH and X-X distribution profiles of wild-type female ES nuclei from day 0 to day 6 of differentiation and of MEFs. Two-probe combination: *Xic* DNA-green (pSxn-FITC) + *Tsx* DNA-red (pTsx-Cy3). DAPI (4′,6′-diamidino-2-phenylindole), blue. Each image is a two-dimensional (2D) representation of 3D image stacks of 0.2 μ z-sections. The distributions display the normalized distances, ND = X-X distance/*d*, where $d = 2 \times (\text{nuclear area}/\pi)^{0.5}$. ND ranges from 0 to 1. Mean distance, open triangle. (**B**) Cumulative frequency curves for X-X pairs at 0.0 to 0.2 ND. *P* (KS test) was calculated in pairwise comparison against day 0. Sample sizes for each experiment (*n*) = 174 to 231. (**C**) X-X distances <0.05 ND were graphed with standard deviations (SD) from three independent experiments. (**D**) Proximity pairing is specific to the *Xic*. X-X distribution profiles for X-linked loci shown in the map. The KS test (*P*) compared *Xic* versus flanking loci. *n* = 166 to 188.

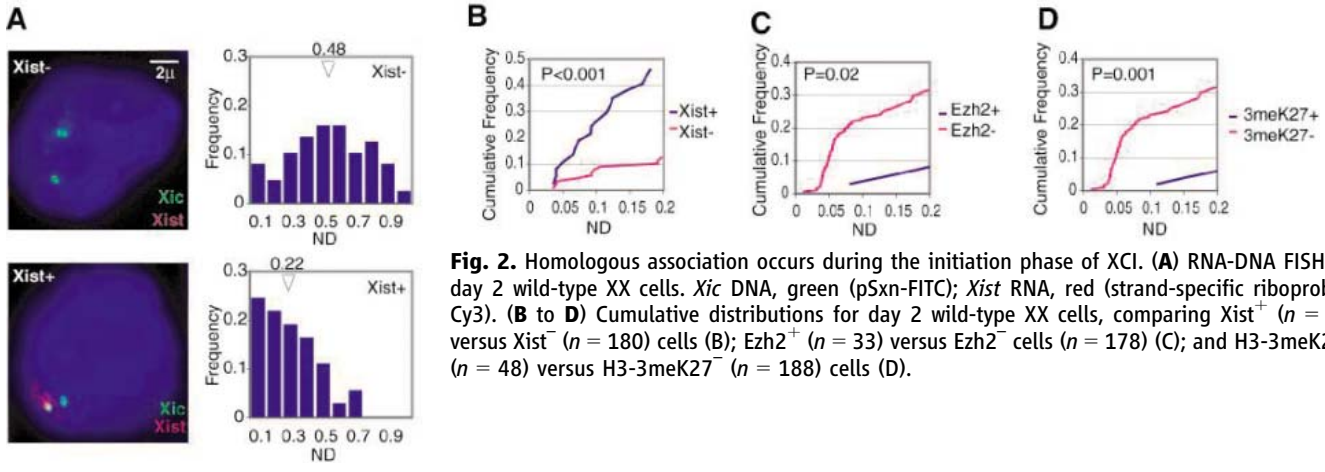

Fig. 2. Homologous association occurs during the initiation phase of XCI. (**A**) RNA-DNA FISH for day 2 wild-type XX cells. *Xic* DNA, green (pSxn-FITC); *Xist* RNA, red (strand-specific riboprobes-Cy3). (**B** to **D**) Cumulative distributions for day 2 wild-type XX cells, comparing $Xist^{+}$ (*n* = 74) versus $Xist^{-}$ (*n* = 180) cells (B); $Ezh2^{+}$ (*n* = 33) versus $Ezh2^{-}$ cells (*n* = 178) (C); and H3-3meK27^{+} (*n* = 48) versus H3-3meK27^{-} (*n* = 188) cells (D).

We therefore tested the relation of trans-interactions to counting and choice, the two earliest steps of XCI, both of which are regulated by *Tsix* and *Xite*. It was previously shown that $Tsix^{+/-}$ mice ($X^{\Delta Tsix}X$) are disrupted for choice and silence only X^{Δ} (*6*, *7*, *10*, *18*), whereas $X^{\Delta Tsix}X^{\Delta Tsix}$ mice are disrupted for both counting and choice (*9*, *11*). *Xite* mutations have similarly affected counting/choice (*8*, *11*). Here, we observed that $X^{\Delta Xite}X$ cells showed a marked delay in X-X association (Fig. 3, A and B, and fig. S5), implying that losing one *Xite* allele is sufficient to partially disrupt pairing. This partial effect correlated with aberrant choice in $X^{\Delta Xite}X$. However, $X^{\Delta Tsix}X$ cells showed the expected frequency of homologous association, indicating that losing one *Tsix* allele does not affect pairing. In contrast, $X^{\Delta Tsix}X^{\Delta Tsix}$ cells showed near-random distributions across all time points (Fig. 3B and fig. S5), which supports the argument that deleting both *Tsix* alleles is required to abolish pairing. Although not statistically significant, day 6 populations showed a slight left shift suggestive of a de-

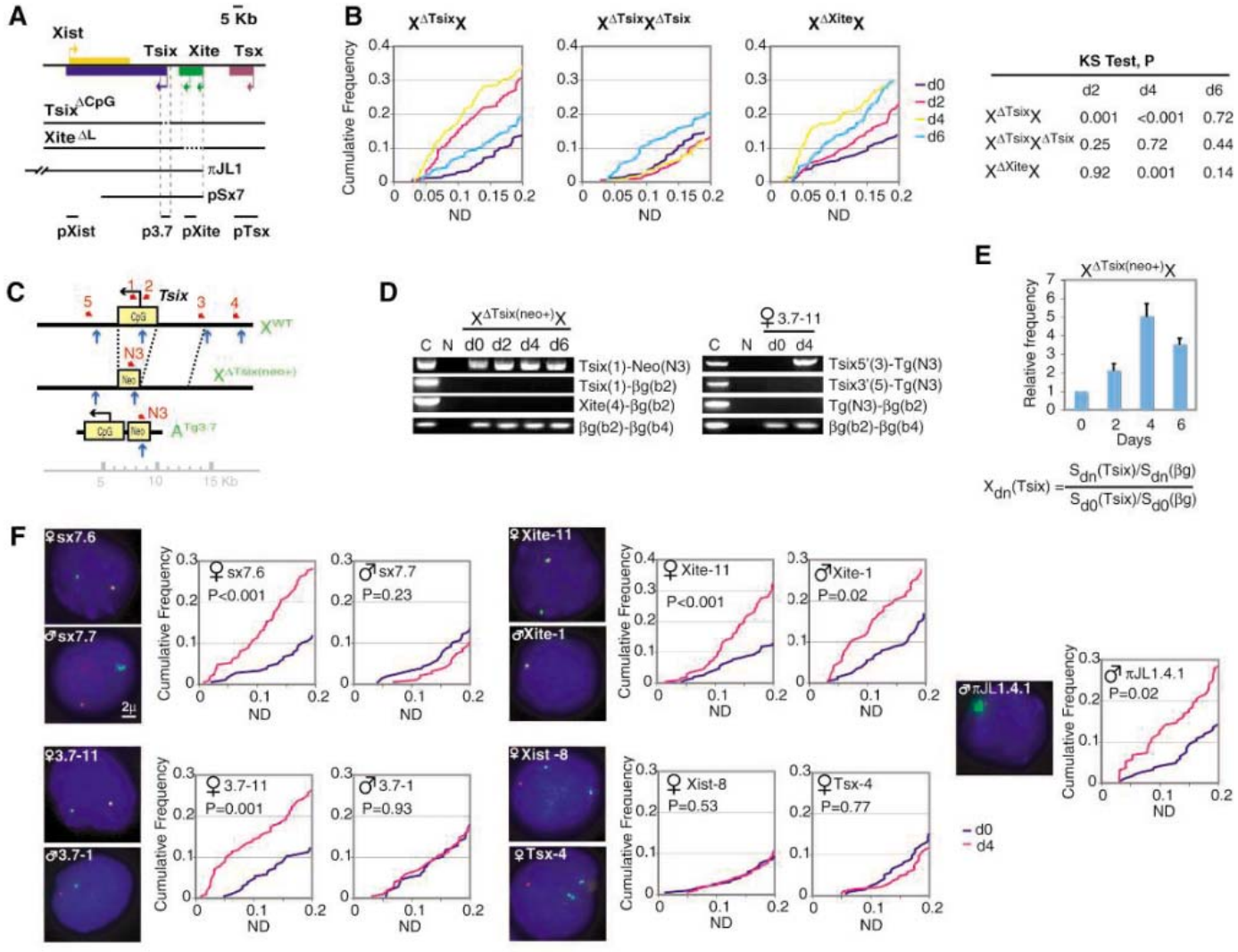

Fig. 3. *Tsix* and *Xite* are necessary and sufficient for X-X pairing. (**A**) Map of the *Xic*, *Tsix*$^{\Delta CpG}$ and *Xite*$^{\Delta L}$, and various transgenes. (**B**) X-X distributions for *Tsix* and *Xite* mutants from day 0 to day 6. n = 181 to 223. KS test compares each curve to the day 0 curve. (**C**) *Tsix* alleles and primers (red) used for 3C analysis. BamHI sites, blue arrow. (**D**) 3C analysis of pairwise interactions in X$^{\Delta Tsix(neo+)}$X cells and p3.7 females. Primers pairs are indicated to the right of gels. C, positive control ligations. All minus-crosslinking (N) and minus-ligation controls were negative. (**E**) Relative pairing frequencies (X) on day n (dn) was normalized to β-globin (βg) and to day 0 values, using the equation shown. S, signal intensity quantitated by densitometry. Average and SD from three independent experiments. (**F**) DNA FISH and X-A distribution curves for transgenic ES cells. The transgene was labeled red by a Neo probe and the X labeled green by a pSx7 probe (for p3.7, pXite, pXist5′, and pTsx cells) or a pTsx probe (for pSx7 cells). The pSx7 partially overlaps the p3.7 and pXite transgenes, but the small overlap makes the signal dim and discernible from the X. For π]L1.4.1, the transgene was labeled green (pSx9 *Xist* fragment) and the X labeled red (pTsx probe). The KS test compared data sets from day 0 versus day 4. n = 170 to 234.

layed or weakened attempt to associate. These data demonstrated that *Tsix* and *Xite* are required for pairing and implied a tight link between pairing and counting/choice.

To learn whether the homologous association represented true physical pairing, we carried out "chromosome conformation capture" (3C) (*19*), whereby two interacting loci can be detected by crosslinking, intermolecular ligation, and polymerase chain reaction. To obtain necessary polymorphisms for 3C, we used the pairing-competent X$^{\Delta Tsix(neo+)}$X line, in which one *Xic* is distinguished by *Neo* (Fig. 3C) (wild-type could not be used because they lack informative polymorphisms within required restriction fragments). Using three distinct primer pairs [Tsix1-N3 (shown), and TSEN2-N1 and Tsix1-N2 (not shown)], we consistently detected physical contact between the two *Tsix* loci, whereas no contacts were observed between various *Tsix* and autosomal controls or the incorrectly oriented Tsix2 primer and N3 (Fig. 3D and fig. S6). The inter-*Tsix* interaction was strongest on day 4 (Fig. 3E), consistent with FISH analysis. Therefore, inter-*Xic* pairing indeed underlies homologous association.

To identify sequences that direct pairing, we introduced *Xic* fragments into ES cells (Fig. 3A and fig. S7) (*11*) and asked whether autosomal insertions could induce de novo X-autosome (X-A) pairing and affect counting/choice. Intriguingly, autosomal pSx7 led to ectopic X-A pairing in females (Fig. 3F), correlating with aberrant counting and XCI initiation in pSx7 females (*11*). By contrast, female *Xist* and *Tsx* transgenics showed no X-A pairing above background (Fig. 3F), consistent with their normal XCI (*11*). Furthermore, male pSx7 transgenics did not exhibit X-A pairing (Fig. 3F), consistent with their normal counting and XCI suppression (*11*).

To dissect specific requirements within pSx7, we tested p3.7, the 3.7 kb *Tsix* fragment deleted in the pairing-incompetent X$^{\Delta Tsix}$X$^{\Delta Tsix}$. p3.7 was remarkably efficient at inducing de novo X-A pairing in XX cells (Fig. 3F), with 3C analysis confirming direct physical interaction between p3.7 and the X (Fig. 3D). The ectopic pairing paralleled the failure of counting/choice

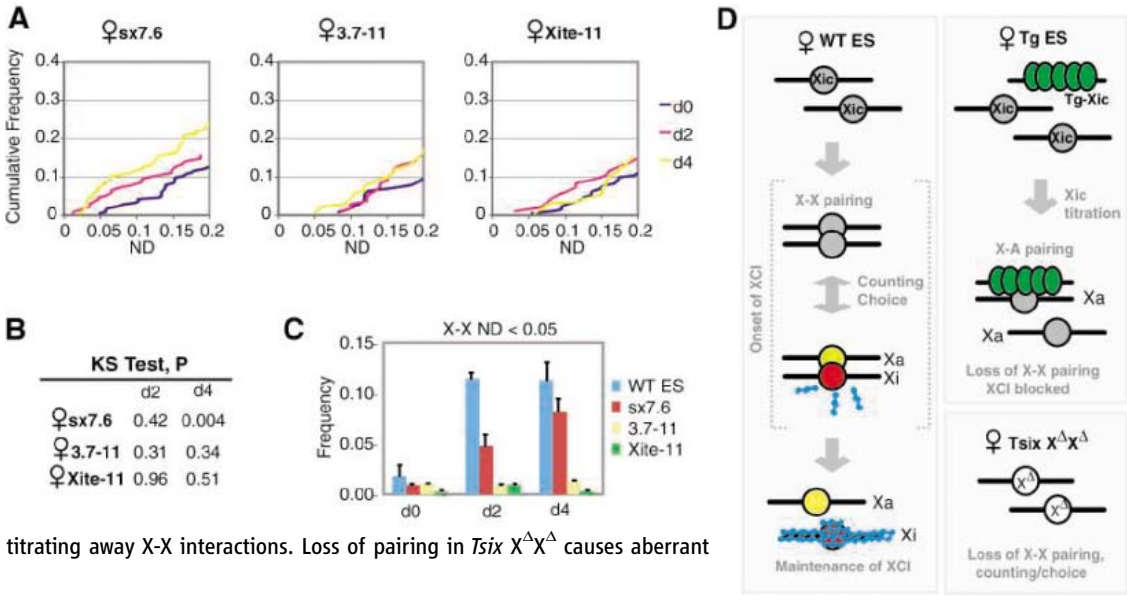

Fig. 4. De novo X-A pairing inhibits X-X pairing. (**A**) Disruption of X-X pairing in female transgenic cells. $n = 177$ to 221. (**B**) KS test compares data sets from day 0 versus day 2 and from day 0 versus day 4. (**C**) Average frequency of X-X pairing with standard deviations from three experiments. (**D**) Model: X-X pairing is required for counting/choice. Allelic crosstalking results in asymmetric chromosome marking (yellow circles, blocked *Xic*; red circle, induced *Xic*) and mutually exclusive designation of X_a and X_i. Blue lines, Xist RNA. Ectopic *Tsix/Xite* transgenes (Tg-Xic) inhibit XCI by titrating away X-X interactions. Loss of pairing in *Tsix* $X^{\Delta}X^{\Delta}$ causes aberrant counting/choice.

and XCI initiation in p3.7 females (*11*). In contrast, p3.7 males did not induce X-A pairing and accordingly did not manifest a counting defect (*11*). We also tested pXite (a 5.6-kb fragment deleted in the pairing-compromised $X^{\Delta Xite}X$) and found efficient X-A pairing (Fig. 3F), consistent with pXite's profound effect on counting/choice (*11*). Interestingly, pXite males could also initiate pairing, although they did not exhibit ectopic XCI (*11*). Because pXite males are thought to lack an X-linked "competence factor" for initiating XCI, we next tested males carrying full-length *Xic* transgenes (*11*) to determine whether pairing and XCI could be achieved together. Indeed, πJL1.4.1 males displayed ectopic X-A pairing (Fig. 3F) and, accordingly, initiated counting/choice and silencing (*20*), further supporting the tight linkage between pairing and XCI initiation. These experiments demonstrated that *Tsix* and *Xite*, with sequences as small as 3.7 and 5.6 kb, are sufficient to recapitulate pairing and that, in turn, pairing is required for the earliest steps of XCI.

In transgenic females, we hypothesize that the failure to initiate XCI may be due to a competitive inhibition of X-X interactions by de novo X-A interactions. Indeed, the frequency of X-X interactions was significantly diminished for pSx7, p3.7, and pXite females as compared with wild-type (Fig. 4A versus Fig. 1B). In pSx7 females, X-X pairing rates were less than X-A pairing rates. In p3.7 and pXite females, X-X pairing appeared to be abolished completely (Fig. 4A and fig. S8), with day 2 and day 4 distribution profiles being indistinguishable from day 0 (Fig. 4B) and <2% of nuclei (background) with ND < 0.05 (Fig. 4C). In contrast, X-X pairing remained robust in pTsx and pXist controls (fig. S8). Therefore, ectopic X-A interactions measurably detracted from endogenous X-X interactions. The frequency of X-X pairing directly predicts the frequency of XCI. We propose that the titration of X-X interactions by ectopic *Tsix/Xite* accounts for the pervasive failure of counting/choice and XCI in transgenic females.

On the basis of this work, we postulate that X-X pairing acts upstream of *Xist* by mediating counting/choice and providing the necessary crosstalk for mutually exclusive XCI. Pairing interactions clearly do not require *Xist* expression. In our model (Fig. 4D), two Xs assume random independent positions in pre-XCI cells and then pair homologously at the onset of XCI, with *Tsix* and *Xite* acting as nucleation centers. The ensuing crosstalking achieves asymmetric marking of one X to become X_a and the other to become X_i. With counting/choice reflecting the binding of a "blocking factor" to the X_a and the competence factor to the X_i (*6*, *11*), pairing ensures that the two factors bind mutually exclusively.

Remarkably, 3.7 kb of *Tsix* or 5.6 kb of *Xite* is sufficient to initiate de novo pairing. Thus, these genes play dual cis-trans roles in XCI by functioning in trans to coordinate pairing/counting/choice and in cis to antagonize *Xist*. These events may take place simultaneously in time and space. Subtle pairing differences between *Tsix* and *Xite* mutants likely reflect length requirements, as indeed $X^{\Delta Xite}X$ shows weaker pairing than $X^{\Delta Tsix}X$, and *Xite* transgenic males pair better than *Tsix* counterparts. Consistent with this, full-length transgenic πJL1.4.1 males not only pair well but also initiate XCI. Why do X-A interactions generally outnumber X-X interactions? The multicopy transgene nature might increase the avidity of the autosome relative to the X. The ability of X-A pairing to inhibit X-X pairing now provides a mechanism for failed XCI in *Tsix/Xite* transgenic females: If pairing were required for proper counting/choice, the failure to pair would pose a specific block to XCI. The proposed regulation by interchromosomal pairing creates a new dimension to the problem of gene regulation and is likely to become a recurrent theme in epigenetic phenomena (*16*, *21*).

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Figs. S1 to S8
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Structure of a DNA Glycosylase Searching for Lesions

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DNA glycosylases must interrogate millions of base pairs of undamaged DNA in order to locate and then excise one damaged nucleobase. The nature of this search process remains poorly understood. Here we report the use of disulfide cross-linking (DXL) technology to obtain structures of a bacterial DNA glycosylase, MutM, interrogating undamaged DNA. These structures, solved to 2.0 angstrom resolution, reveal the nature of the search process: The protein inserts a probe residue into the helical stack and severely buckles the target base pair, which remains intrahelical. MutM therefore actively interrogates the intact DNA helix while searching for damage.

One of the most formidable needle-in-a-haystack challenges in biology is that faced by DNA glycosylases as they locate and excise damaged DNA nucleobases embedded in a greater than millionfold excess of undamaged DNA (*1*, *2*). The challenge is compounded by the fact that many damaged bases targeted by DNA glycosylases are only subtly different from their normal counterparts; for example, the highly mutagenic lesion 8-oxoguanine (oxoG) differs from G by only two atoms, and these modest alterations give rise to no major structural or energetic aberrations in the DNA helix (*3*–*5*). Because DNA glycosylases consume no biochemical energy during the search for damage, they must rely solely on thermally driven translocation along vast expanses of the genome. These constraints on the search process, plus the deleterious biologic effects of the failure to repair genotoxic lesions, have forced DNA glycosylases to evolve an exceptionally efficient means of interrogating DNA. Despite widespread speculation (*1*, *2*, *6*, *7*), the biophysical underpinnings of the process remain poorly understood (*8*, *9*).

The structure of the lesion recognition complex (LRC) comprising MutM, a bacterial DNA glycosylase specific for oxoG, bound to oxoG-containing DNA has revealed that MutM extrudes the damaged nucleotide from the DNA helix and inserts it into an active-site pocket on the enzyme (*10*) (Fig. 1A). Despite having no structural relationship to MutM, the eukaryotic functional counterpart of MutM, Ogg1, uses the same extrahelical strategy for oxoG recognition and repair (*11*). Indeed, an extensive body of diverse LRCs (*7*, *12*, *13*) strongly suggests that all DNA glycosylases operating on single-base lesions in the genome perform extrahelical base excision.

These studies leave unresolved the question of whether these enzymes search for lesions by actively extruding every base from DNA and presenting it for inspection to the active site. The energetic demands of such an extrahelical search would seem prohibitive. Alternatively, the active sites of DNA glycosylases might simply capture lesion bases that have undergone spontaneous extrusion from the DNA helix. Evidence has been presented that uracil DNA glycosylase uses such an extrahelical capture mechanism (*9*), but such a mechanism has not been demonstrated for other systems. Yet another plausible mechanism for lesion searching entails recognition of lesions by the DNA glycosylase while they still lie within the DNA helix (*14*), thus triggering extrusion and presentation to the active site. This option, intrahelical lesion recognition, is in many respects the most kinetically tenable, yet the structural basis for such recognition remains obscure.

Recently, intermolecular disulfide cross-linking (DXL) technology has been used to capture human 8-oxoguanine DNA glycosylase (hOGG1) in the act of extruding a normal nucleobase from DNA (*8*). To capture earlier intermediates in DNA interrogation, we focused on the bacterial counterpart of hOGG1, MutM, which has been the subject of extensive biochemical (*14*–*17*) and structural (*10*, *18*–*21*) analysis. Here, we describe the use of DXL to obtain multiple structures of MutM performing an invasive interrogation of an undamaged DNA helix. These structures illuminate an early stage of the search process and suggest that oxoG lesions are located by intrahelical interrogation.

We identified several potential sites for DXL in the protein-DNA interface of the *Bacillus stearothermophilus* MutM LRC (Fig. 1) (*10*). MutM variants containing single cysteines at the relevant positions were created, and oligonucleotides were synthesized (*22*–*25*) each containing a single thiol tether attached to the corresponding backbone phosphates (Fig. 1B). These thiol-tethered oligonucleotides were incubated with the respective MutM variants, and the extent of cross-linking was analyzed by SDS–polyacrylamide gel electrophoresis under non-reducing conditions (fig. S1). The combination that exhibited the most favorable time course (fig. S1) and furnished the cross-linked product in the highest yield and purity was Q166C/p9′ with a two-carbon (C2) tether. This complex (hereafter referred to as interrogation complex 1, IC1) was therefore selected for further investigation.

Diffraction-quality crystals of IC1 were obtained after substantial modifications of the conditions used to grow crystals of the LRC (*10*). Nonetheless, the IC1 crystals, which diffracted to 2.0 Å resolution, belonged to the same space group and had approximately the same unit cell dimensions as those of the LRC (see table S1 for data collection and refinement statistics). The structure of IC1 was solved by molecular replacement using the coordinates of the protein from the structure of the *B. stearothermophilus* MutM LRC (*10*).

Our cross-linking scheme was designed to elucidate the structural details of MutM interrogating a G:C base pair at the same position [Fig. 2, base pair 8 (bp8)] that was previously occupied by the oxoG:C base pair in the LRC. Surprisingly, inspection of the IC1 structure revealed that the protein was not interrogating the intended target G:C base pair, but instead had positioned itself on the DNA so as to interrogate the A:T base pair at bp9 (Fig. 2B, cyan). Evidently, the disulfide cross-link incorporated into this protein-DNA interface still allows sufficient freedom of movement for MutM to reposition itself by a full base pair step along DNA.

In the LRC, the oxoG lesion is extruded into the extrahelical enzyme active site, and the DNA helix is penetrated by three key residues on the enzyme (Figs. 2E and 3A). The aromatic ring of Phe^{114} is wedged into the helical stack to enforce a sharp bend in the DNA. The side chain of Arg^{112} extends into the space vacated by oxoG and forms hydrogen bonds with the Watson-Crick face of the estranged C. Met^{77} also fills the void in the DNA helix, making van der Waals contacts with the nucleobase on the 3′ side of the lesion and with the sugar of the nucleotide on the 5′ side.

The nature of the protein-DNA interaction in IC1 differs in one major respect from that previously determined for any DNA glycosylase complex, in that the target base pair in DNA is completely intact and fully intrahelical. Although intact, the target A:T base pair is buckled, probably as the result of the forced insertion of Phe^{114} into the helix directly above the Watson-Crick hydrogen bonds of the A:T pair (Fig. 3B). Whereas Phe^{114} serves an analogous role in IC1 and the LRC, Arg^{112} and Met^{77} do not. With no vacant space in the helix, the side chain of Arg^{112} is retracted from the DNA and points downward into the minor groove space, forming hydrogen bonds to the O4′ of G10 and G11 and the N3 atom of G10. The side chain of Met^{77} is rotated and no longer makes the same van der Waals contacts with the DNA, but the methyl group of Met^{77} does contact the sugar moiety of the target A. The loop containing Met^{77} is retracted from the DNA surface by ~1 Å relative to its position in the LRC, and this presumably serves

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to weaken the interactions between this residue and the DNA. The only notable conformational change in the protein is the loss of ordered structure in the prominent surface loop that makes four hydrogen bonds to the extrahelical oxoG in the LRC (*10*); in IC1 and in all other structures of interrogation complexes presented here (see below), this loop is completely disordered. This oxoG recognition loop is also disordered in all structures of MutM bound to postexcision intermediates (or analogs thereof) in the base-excision repair pathway (*19*–*21*) that no longer possess an oxoG nucleobase.

In an attempt to encourage MutM to interrogate the G:C base pair at position 8, we moved the cross-link attachment point from p6 to p5. The structure of the resulting complex revealed a pronounced overall helical distortion in the DNA that enabled MutM to continue interrogating the A:T base pair at position 9 (+4 register) (*26*). This observation indicates a preference on the part of MutM to interrogate A:T base pairs rather than G:C. To test the notion that the induced duplex distortion results from an aversion to interrogate a G:C pair, we mutated bp8 to A:T while maintaining the tether attachment point at p5 and solved the structure of the cross-linked complex (hereafter referred to as interrogation complex 2, IC2) to 2.0 Å resolution. In this structure (Fig. 2C) (fig. S2), MutM adopts the +3 register on DNA and interrogates the A:T base pair at position 8 in the duplex. The conformation of the DNA flanking the target base pair is typical B form. Thus, changing the G:C base pair at position 8 to A:T alleviates the aversion to interrogating bp8 and relieves the attendant helical distortion. Even though MutM is slid by one base pair along the duplex in IC1 versus IC2, the overall helical structure and local interactions at the site of helix penetration by Phe[114] are remarkably similar in the two complexes (Fig. 2F) (figs. S3 and S4).

To capture MutM interrogating a G:C base pair, we swapped the G:C and A:T base pairs at bp7 and bp9 in the IC1 DNA, thereby presenting the protein with a stretch of DNA containing only G:C pairs in the +2, +3, and +4 registers. We crystallized this complex (IC3) and solved its structure to 2.05 Å resolution. Inspection of the structure of IC3 reveals MutM to be bound over DNA in the +3 register, interrogating a fully intrahelical G:C base pair at position 9 in the duplex (Figs. 2D and 3C) (fig. S3). Notwithstanding the aforementioned aversion of the protein to interrogate G:C base pairs, the resulting structure (Fig. 3C) is strikingly similar to that of the A:T interrogation complexes, with a severely buckled base pair at the site of helix penetration by Phe[114], and with Arg[112] and Met[77] lying in wait (see fig. S4 for details of the buckling parameters).

The structure of the LRC (*10*) revealed a duplex structure with a sharp bend (~80°) at the site of the lesion, enforced through extensive interactions with the DNA backbone of both the lesion-containing and non–lesion-containing strands. To what extent are these interactions maintained or disturbed in the interrogation complexes? To answer this question, we solved structures of control cross-linked LRCs (E3Q, Q166C MutM) having oxoG:C in place of the non–lesion-containing target base pair in IC1 and IC3 (control complex 1, CC1), or in IC2 (CC2) (figs. S3 and S5). The structure of CC2 superimposes on the LRC with an overall heavy-atom root mean square deviation (RMSD) of 0.250 Å, and CC2 exhibits no apparent structural distortion at the site of tether attachment; CC1 also compares similarly well with the same complex lacking a cross-link (*27*), thus demonstrating the absence of any discernible cross-link–induced structural perturbation.

The overall positioning of DNA with respect to the surface of MutM, and the extent and locus of DNA bending, is so similar in the LRCs and the interrogation complexes (Fig. 2, E and F) (fig. S3) that a close inspection is required to discern the differences between them. The pronounced overall similarity of IC1,

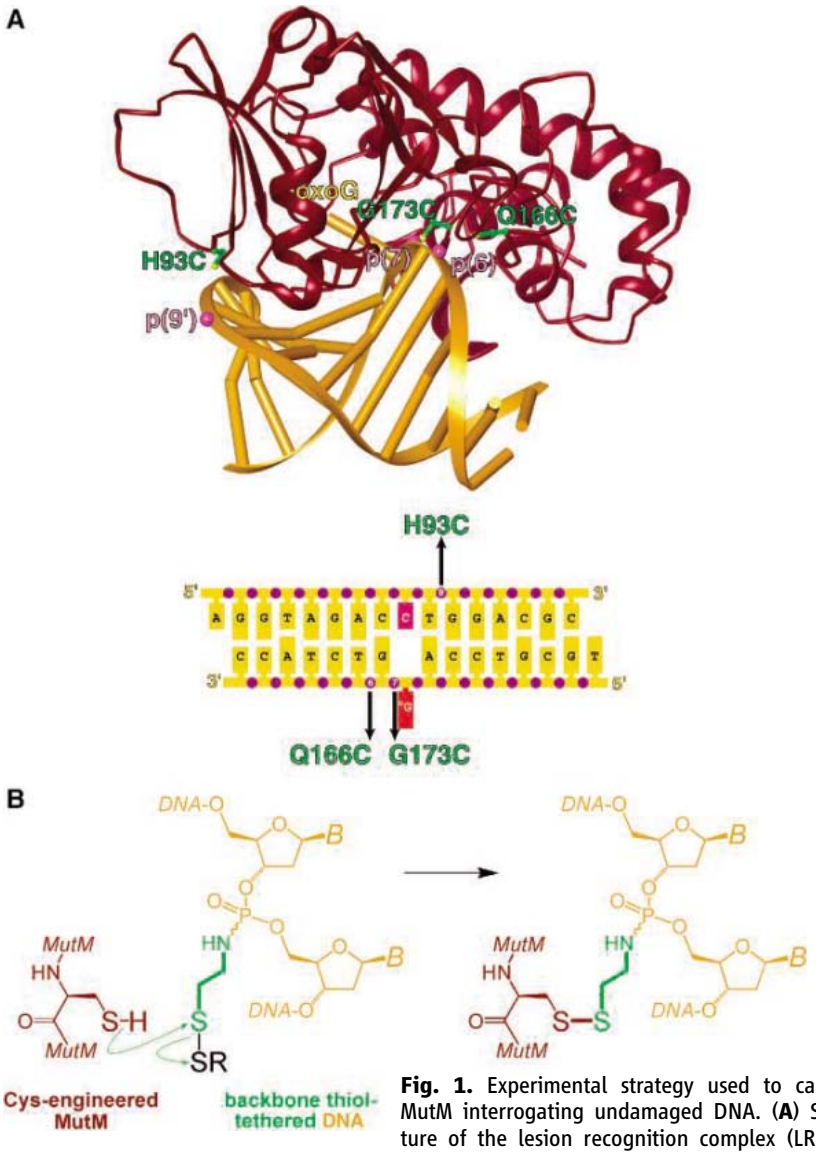

Fig. 1. Experimental strategy used to capture MutM interrogating undamaged DNA. (**A**) Structure of the lesion recognition complex (LRC) of MutM (crimson) bound to oxoG-containing DNA (gold). The side chains of Cys residues introduced individually are shown in green, with nearby backbone phosphates shown in magenta. The schematic sequence diagram below the structure illustrates the relationship of the cross-linking sites in DNA to the positions of the oxoG lesion and its complementary C (the "estranged" C). Abbreviations for amino acids: C, Cys; G, Gly; H, His; Q, Gln. (**B**) Structure of the N-thioalkyl phosphoramidate moiety substituted for a backbone phosphate (thiol-containing tether in green, DNA backbone in yellow); wavy line indicates a mixture of two diastereomers at phosphorus. Only the C2 linker is shown; homologation by the insertion of one or two additional $-CH_2-$ groups into the linker gives C3 and C4, respectively. Also shown is the chemical structure of the starting materials and products of the cross-linking reaction; curved arrows denote electron flow in the cross-linking reaction.

IC2, and IC3 with MutM LRCs supports the notion that the interrogation complexes described here represent an early intermediate (*28*) in the base extrusion pathway used by MutM to search for lesions in DNA and present them to the extrahelical active site.

Closer inspection of the structures, however, reveals a substantial but highly localized structural reorganization concomitant with lesion recognition. The energetically favorable (*29*) transition from a nonlesion interrogation complex to a lesion-specific recognition complex entails conformational rearrangements that are localized almost exclusively to the interface between MutM and the target strand. Most noteworthy is the wholesale replacement of multiple direct backbone contacts to the target strand in CC2, CC1, and LRC with an extensive array of indirect contacts involving ordered bridging water molecules (Fig. 4) (fig. S6). The swiveling motion about phosphates flanking the lesion nucleoside that accompanies helical extrusion appears to reposition them from a conformation favoring water-mediated backbone contacts to one favoring direct contacts. The oxoG appears to exit the duplex from the minor groove side, opposite to what has been proposed for hOGG1 (*8*). Although the precise details of the contact repertoire differ among IC1, IC2, and IC3, all share the overall similarity of using an extensive array of water-mediated contacts, plus a few direct contacts, instead of the mostly direct contacts seen in the lesion-specific complexes. In this respect, the transition from an interrogation complex to a lesion-specific complex in the case of MutM is accompanied by the same decrease in solvation at the protein-DNA interface that was documented for sequence-specific DNA binding proteins bound to noncognate versus cognate DNA sequences (*30–34*). These interfacial waters most likely provide a form of lubricant to facilitate fast sliding of the protein along normal DNA.

Notwithstanding the local differences noted above in protein-DNA interactions between the structures of MutM bound to damaged DNA and to non–lesion-containing DNA (IC1, IC2, and IC3), one prominent feature emerges as being common to all of these complexes. In these and all other structures of MutM and its orthologs bound to DNA (*10*, *18–21*), Phe^{114} is inserted into the DNA duplex, and this is accompanied by nearly complete loss of helix stacking at the site of intercalation. Furthermore, in normal DNA, insertion of Phe^{114} is associated with the buckling of a target base pair at the intercalation site. Buckled base pairs are intrinsically less stable than canonical pairs; hence, Phe^{114} can be seen to cause a highly localized destabilization of the target base pair. This implies that Phe^{114} can act as a sensor for the deformability of base pairs in DNA. Stated otherwise, intercalation of Phe^{114} imposes a specific structural and energetic test on the tar-

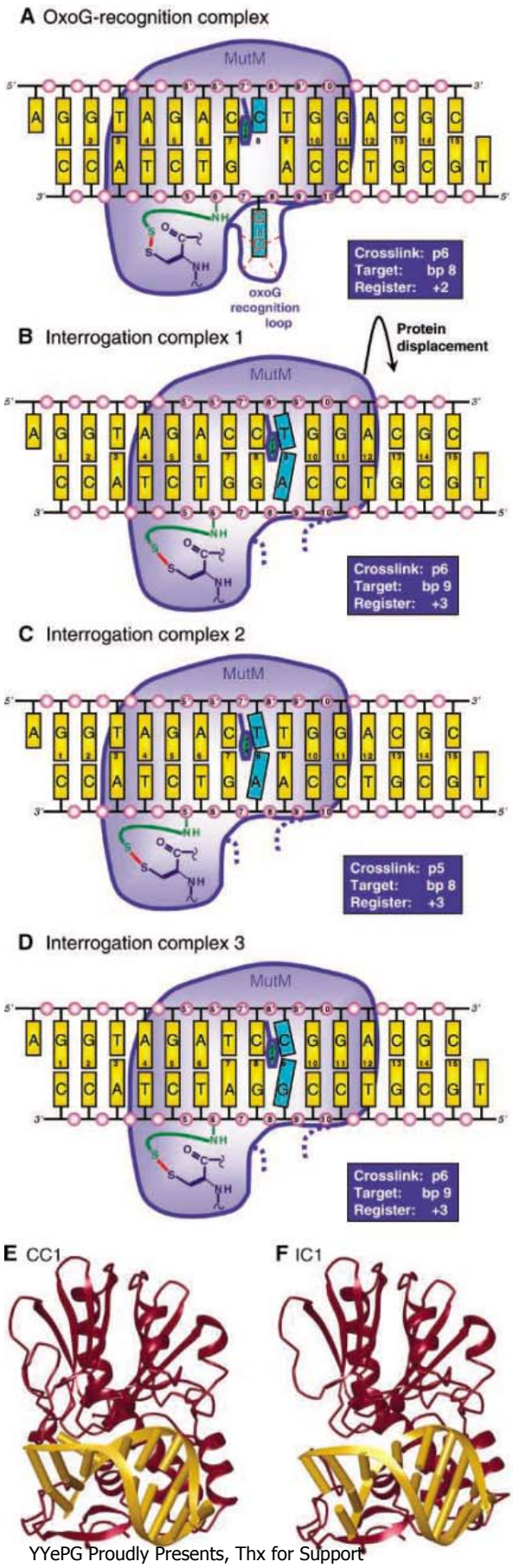

Fig. 2. Schematic representation of MutM-DNA complexes. (**A**) The MutM LRC used as the basis for the design of the cross-linking system. (**B** to **D**) Interrogation complexes showing the positioning of MutM over the DNA duplex, with the target base pair in aqua. The side chain of the helix-probe residue Phe^{114} is indicated. The numbering system for the base pairs and backbone phosphates is as indicated. The curved green line denotes the thiol-bearing tether engaged in a cross-link to Cys^{166}. Each blue box indicates the site of tether attachment to DNA, the position of the target base pair, and the separation between them, here referred to as the register. Dashed blue lines indicate the lack of order in the oxoG recognition loop. (**E** and **F**) Overall view of complexes CC1 (E) and IC1 (F). CC1 is a lesion recognition complex (LRC) formed by disulfide cross-linking between MutM and oxoG-containing DNA. IC1 is the corresponding interrogation complex having MutM cross-linked to non–lesion-containing DNA. Blue box denotes the target base pair, which is disrupted in (E) and intact but buckled in (F).

get base pair in DNA. Although the destabilizing effect of Phe[114] insertion may well be below the magnitude that can be observed in nuclear magnetic resonance experiments measuring imino proton exchange rates (*9*), they could nonetheless make an important contribution to the lesion-searching process and base extrusion pathway. It is noteworthy that in the structure of unliganded MutM, both the side chain of Phe[114] and the protein backbone in the region surrounding this residue are highly constrained conformationally, as judged by inspection of the structure (fig. S7) and by the lower temperature factors (*B* factors) for this region than for the average residue. Insertion of Phe[114] thus appears to arise as an unavoidable consequence of formation of an intimate DNA-bound complex by MutM. The structures of LRCs bearing DNA glycosylases from diverse superfamilies (fig. S8) all reveal a helix-intercalating probe residue, which suggests that this strategy may be widely used to test the structure and energetics of base pairs in DNA while searching for lesions.

The present structures suggest a means by which MutM, and by extension hOGG1, may be able to sense the presence of oxoG residues in an intact DNA helix (intrahelical lesion recognition). Even though oxoG:C base pair has no discernible effect on the conformation of duplex DNA (*3*, *4*) and has only slightly weaker pairing than A:T (*5*), perhaps oxoG:C behaves differently from A:T and G:C when subjected to interrogation by the intercalating probe residue. Taking into account the fact that the lesion search process is Brownian and therefore highly redundant, it is less critical that the enzyme recognize an oxoG lesion upon every encounter, because the enzyme will have many additional opportunities to identify the lesion. What is important is that the enzyme minimizes the incidence of a time-intensive interrogation of nonlesion base pairs in normal DNA. This realization leads to two additional important notions: (i) Negative selection against extruding bases from normal DNA may be at least as important for lesion recognition as positive selection for extrusion of oxoG lesions. As shown here, MutM clearly exhibits an aversion to intercalative probing of G:C base pairs, suggesting at least one way in which negative selection

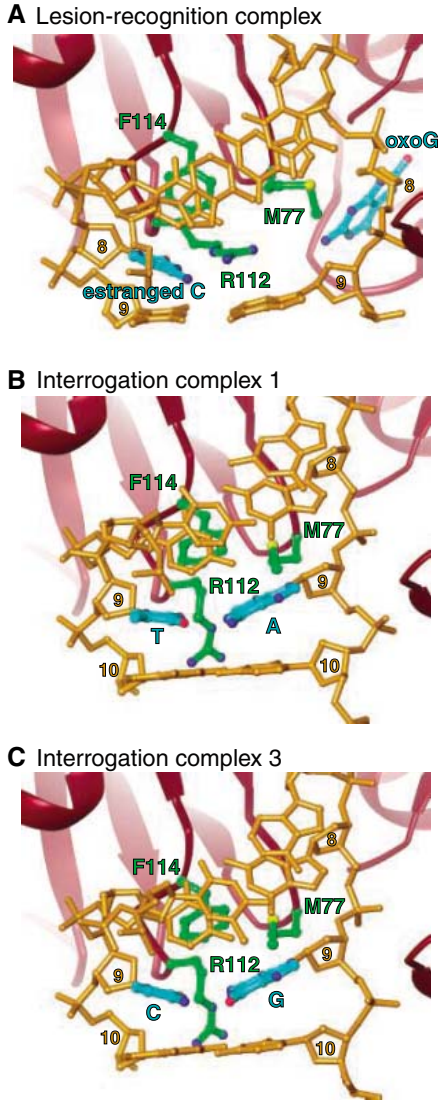

Fig. 3. Close-up views of key interactions in the MutM-DNA interface. (**A**) The control cross-linked LRC CC2; (**B**) IC1; (**C**) IC3. The protein backbone is in a crimson ribbon representation, with the side chain of key residues shown in green. The DNA is in a yellow framework model, with the target base pair in aqua. The target base pair is broken in (A) by extrusion of oxoG from the helix and insertion into the active-site pocket; the target base pair in (B) and (C) is intact but severely buckled. Abbreviations for amino acids: F, Phe; R, Arg; M, Met.

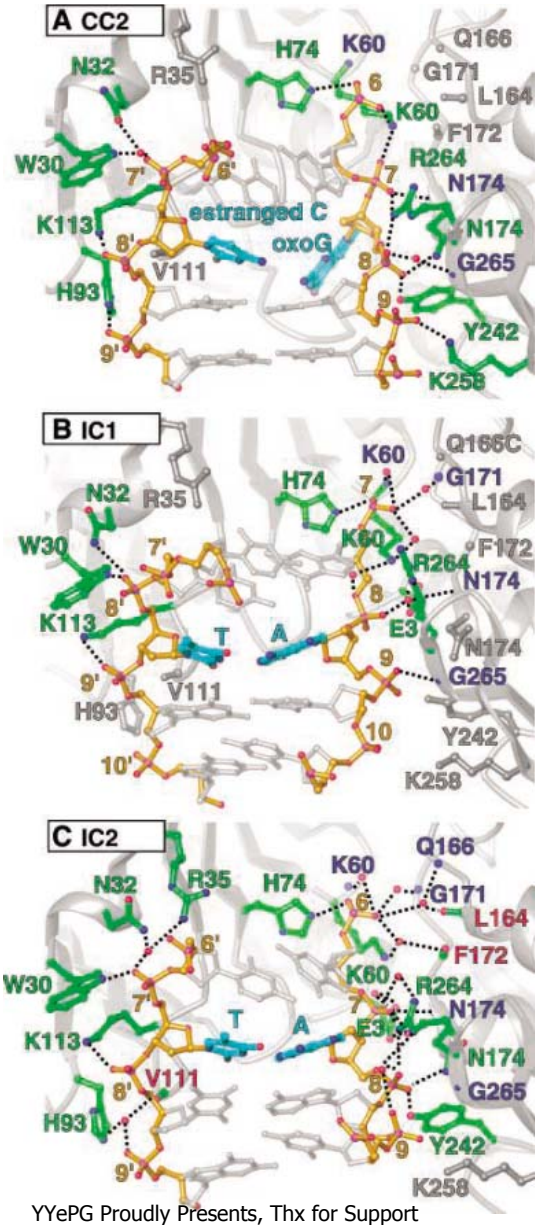

Fig. 4. Direct and water-mediated interactions between MutM and the DNA backbone in the control LRC CC2 (**A**) and interrogation complexes IC1 (**B**) and IC2 (**C**). Dashed lines indicate hydrogen bonding interactions among backbone phosphates in DNA, ordered waters (red spheres), and residues in MutM. The side chains of amino acid residues are shown in green with the numbers colored according to which moiety on the amino acid is involved in the contact: green, side chain; blue, backbone amide NH; red, backbone amide carbonyl; gray, no contact in that particular complex.

can be achieved. (ii) Lesion discrimination seems to result from a kinetic preference to extrude oxoG residues from the DNA helix, relative to normal bases. Should the enzyme occasionally make a mistake and attempt to present a normal base to the active site, that poses no danger, as we have shown, in the case of hOGG1, that the active site has an exquisite ability to discriminate thermodynamically in favor of oxoG (*8*).

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Supporting Online Material

www.sciencemag.org/cgi/content/full/311/5764/1153/DC1
Materials and Methods
Figs. S1 to S8
Table S1

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Coherent Sign Switching in Multiyear Trends of Microbial Plankton

William K. W. Li,* W. Glen Harrison, Erica J. H. Head

Since the 1990s, phytoplankton biomass on the continental shelf of Nova Scotia and in the Labrador Sea has undergone sustained changes in the spring and fall, which are accompanied by changes in bacterioplankton that are dampened in amplitude but coherent in the direction of change. A reversal of trend in biomass change, so-called sign switching, occurs both in time and in space. Thus, whenever (spring or fall) and wherever (Scotian Shelf or Labrador Sea) phytoplankton increase or decrease, so also does bacterioplankton. This tandem sign switch indicates coupling of the trophic levels at a multiyear time scale and contributes to an ecological fingerprint of systemwide forcing.

Sustained monitoring of large ocean ecosystems often reveals systematic changes in phytoplankton abundance. These have been ascribed to systemwide influences such as climate change (*1*) and removal of top predators (*2*). Changes at one trophic level may cascade up or down the food web, and the effects may be discerned when other trophic levels are also monitored (*3*). A large proportion (about 50%) of primary production is routed through the microbial loop in which heterotrophic bacterioplankton assimilate or respire the substrates originating from phytoplankton (*4*). It might thus be inferred that a long-term change in phytoplankton would lead to a concomitant change in bacterioplankton, but we are not aware of any direct evidence at the appropriate time scale.

Here, we show that since the 1990s, phytoplankton biomass on the continental shelf of Nova Scotia and in the Labrador Sea has undergone sustained changes that are accompanied by changes in bacterioplankton, dampened in amplitude but coherent in the direction of change. A reversal of trend in biomass change, so-called sign switching (*5*), occurs both in time (between seasons) and in space (among locations), and the two trophic groups switch sign in tandem. Although heterotrophic and photoautotrophic plankton interact at the short scales of microbial generation times and cellular distances, the ecological relationship between these two trophic groups (at the large scales of a decade and ocean shelves) appears responsive to systemwide forcing.

The Scotian Shelf (SS) has been sampled every spring (April and May) and every fall (October) since 1997 along a western (WSS), a central (CSS), and an eastern (ESS) section, with seven stations on each section comprising the core element of the Canadian Atlantic Zone Monitoring Program (*6*). Additionally, at a single CSS station (HL2, 44.27°N, 63.32°W), there is supplementary sampling once every 2 weeks to delineate higher frequency events. The Labrador Sea has been sampled every spring or early summer (May to July) since 1994 on 28 stations along a section (*7*) starting from Hamilton Bank on the Labrador Shelf (LS), through the central deep Labrador Basin (LB), and ending at Cape Desolation on the Greenland Shelf (GS). At each of the 49 hydrographic stations (fig. S1), we monitor chlorophyll concentration and bacterioplankton abundance (*8*) from the sea surface to 100 m and compute depth-integrated standing stocks of both biotic components. Semimonthly values of surface chlorophyll are also computed throughout the study region with the use of satellite ocean color imagery (*8*).

The biweekly record of depth-integrated chlorophyll concentration at HL2 shows repeated cycles of a major bloom in spring and a minor bloom in fall (Fig. 1A). Over 6 years, average spring chlorophyll (March to May) has been increasing at 14% per year while average fall chlorophyll (September to November) has been decreasing at –9% per year. The same directions of change are also evident in the remotely sensed record of surface chlorophyll (Fig. 1B), abundance of diatoms (Fig. 1C), and abundance of dinoflagellates (Fig. 1D), but with only weak or no statistical significance in all of them (table S1).

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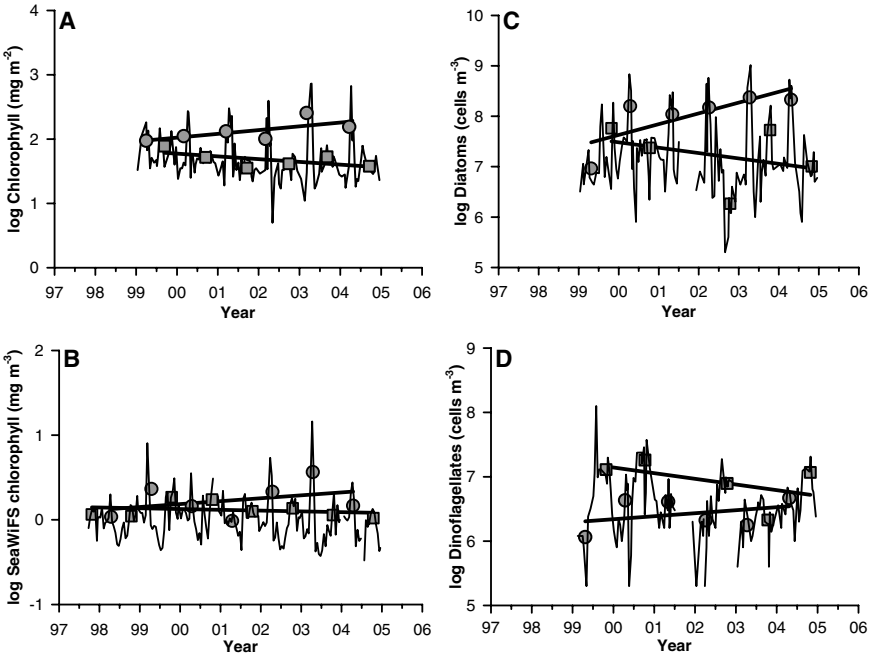

Fig. 1. Time series of phytoplankton at station HL2 in CSS: (**A**) log depth-integrated chlorophyll concentration, (**B**) log SeaWiFS satellite surface chlorophyll concentration, (**C**) log diatom cell concentration, and (**D**) log dinoflagellate cell concentration. Semimonthly time series are indicated by fluctuating lines. Annual spring values are averaged from measurements in March to May (gray circles); annual fall values are averaged from measurements in September to November (gray squares). Multiyear trends in spring and fall are indicated by linear regression.

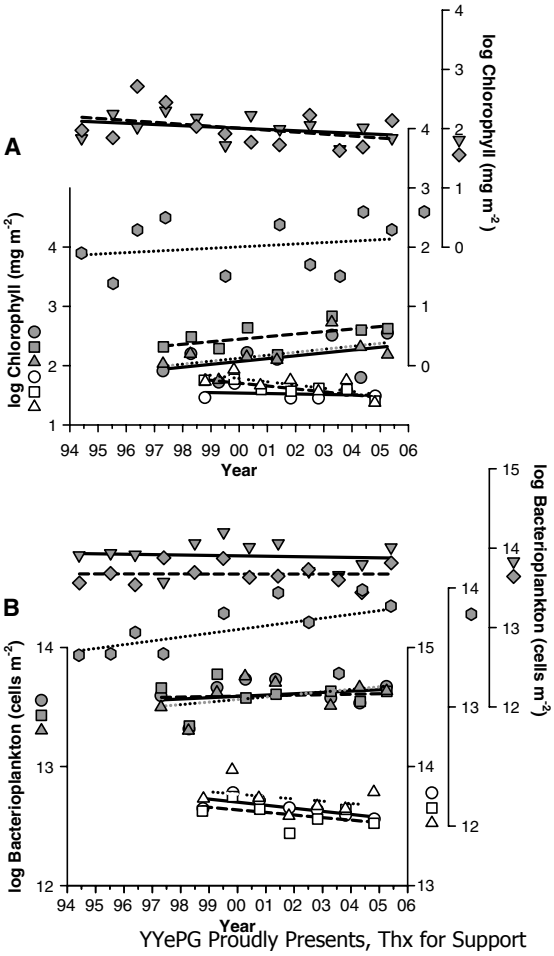

Fig. 2. Time series of log depth-integrated plankton variables showing station averages in various subregions. (**A**) Chlorophyll concentration. (**B**) Bacterioplankton abundance. Code for symbols: CSS spring, gray circle; ESS spring, gray square; WSS spring, gray triangle; CSS fall, white circle; ESS fall, white square; WSS fall, white triangle; LB spring, gray inverted triangle; LS spring, gray diamond; GS spring, gray hexagon. The appropriate ordinate for each time series in (A) and (B) is indicated by matching symbols on the *y* axes.

On the Scotian Shelf as a whole, depth-integrated chlorophyll has increased in the spring at about 12% per year and decreased in the fall at about –6% per year (Fig. 2A). With the exception of CSS in the fall, the changes in chlorophyll are significant ($P < 0.10$) at all other times and places (table S1). Analysis of covariance indicates the statistical equality ($P > 0.10$) of the rate of change for the stations within each subregion (table S1).

Independent support of these trends is provided by high-frequency satellite data. Season averages calculated from semimonthly composites of surface ocean color (Fig. 3, A to C) confirm the spring increases (average 9% per year) and fall decreases (average –2% per year) on the Scotian Shelf. Notwithstanding the weak statistical trends in individual time series, there is a strong correlation ($r = 0.82$, $P = 0.007$) between change indicated by in situ observations and remote observations (Fig. 4A). This correlation provides some confidence that the multiyear trends established by low-frequency sampling are not obscured by aliasing. Although it might appear remarkable that a semiannual sampling schedule could yield significant trends over a relatively small number of years, it should be noted that each in situ value for a particular subregion at any year (Fig. 2) embodies a great deal of environmental averaging by depth and stations. Depth-integrated station averages are tantamount to smoothing over vertical and horizontal space. Local contingencies are subsumed to better reveal any underlying trend. Strong local forces such as short-term wind-driven upwelling events (*9*) can overwhelm a small systematic trend, but spatial averaging generates more confidence in purported temporal change.

As a whole, these results point to a shelfwide trend of "spring-up and fall-down" in the intensity of chlorophyll. Historical measurements extend the springtime pattern backward. For example, depth-integrated chlorophyll on the Halifax Line in May 1974 averaged 33 ± 16 mg m^{-2} (*10*), a value considerably lower than the present-day spring average of 153 ± 115 mg m^{-2}. Additionally, data from institutional archives (*2*) indicate that at surface depths (<10 m), springtime chlorophyll on the shelf as a whole was significantly lower ($F_s = 7.99$, $P = 0.0075$; fig. S2A) from 1974 to 1981 (0.89 mg m^{-3}) than from 1996 to 2005 (2.0 mg m^{-3}). Finally, the green color index from the Continuous Plankton Recorder (*11*) also indicates significantly lower ($F_s = 14.1$, $P = 0.0005$; fig. S2B) springtime values from 1961 to 1976 (0.19 units) than from 1991 to 2003 (0.34 units). For reasons of methodology and the nature of phytoplankton, the color index cannot reliably predict chlorophyll concentrations (*12*, *13*). Furthermore, because the quantities are related to each other differently according to season (*13*), any comparison between spring and fall color indexes would not inform us of seasonal chlorophyll differences.

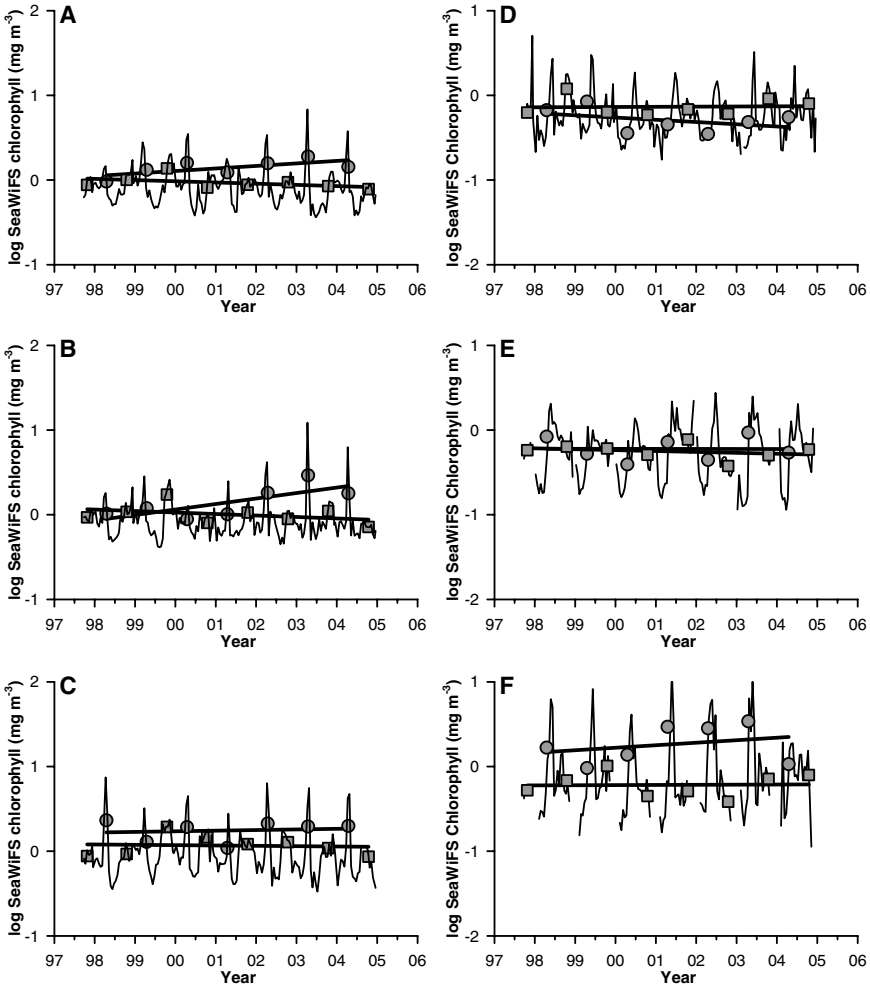

Fig. 3. Time series of log SeaWiFS satellite surface chlorophyll concentration in various subregions: **(A)** Central Scotian Shelf, **(B)** Western Scotian Shelf, **(C)** Eastern Scotian Shelf, **(D)** Labrador Shelf, **(E)** Labrador Basin, and **(F)** Greenland Shelf. Lines and symbols are as in Fig. 1B.

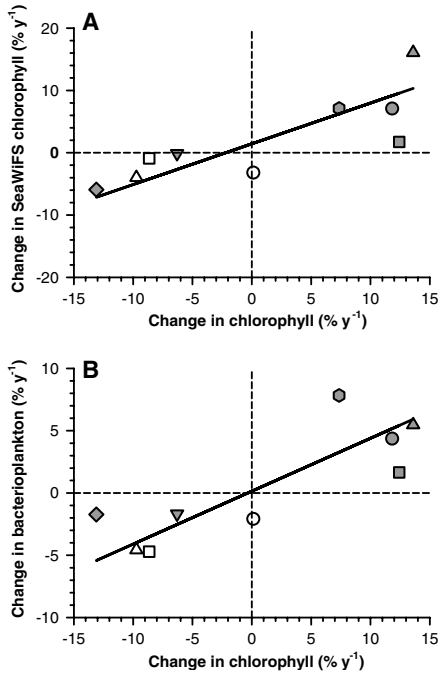

Fig. 4. Quadrant plots comparing multiyear change. **(A)** SeaWiFS satellite surface chlorophyll concentration versus in situ depth-integrated chlorophyll concentration. **(B)** Depth-integrated bacterioplankton abundance versus depth-integrated chlorophyll concentration. Code for symbols is as in Fig. 2.

On an annual basis, the opposing seasonal trends in chlorophyll on the Scotian Shelf largely cancel each other, yielding only small net changes (–1 to +3% per year) that are not statistically significant ($P > 0.50$). Elsewhere in other coastal oceans, a significant average increase (10% per year) of annual chlorophyll in recent years has been recorded and has been described as a possible response to enhanced coastal upwelling or anthropogenic influences (*14*). In the present regime on the Scotian Shelf, it can be said that apparent annual stability is the result of diverging but partially compensating changes in spring and fall. This small divergence could conceivably lead to an alternate regime if it were sustained for many years. Altered phytoplankton phenology has already been shown to affect higher trophic levels (*15*, *16*).

Turning now to the Labrador Sea subpolar gyre, we have a slightly longer record of observations here (1994 to 2005) for the spring, but none at all for the fall. Only on the GS has chlorophyll increased (Fig. 2A). Elsewhere in the gyre, there is a countertrend. Chlorophyll has undergone a significant springtime decrease of –13% per year on the LS and –6% per year in the LB (Fig. 2A). The direction of springtime change extracted from the high-frequency satellite record of ocean color matches in situ chlorophyll change in each subregion (Fig. 3, D to F, and Fig. 4A).

The opposite trends of chlorophyll in different seasons and subregions provide an opportunistic test of the hypothesized long-term linkage between phytoplankton and bacterioplankton. This is an experiment provided by nature. On the Scotian Shelf where there is "spring-up and fall-down" of chlorophyll, the same directional changes appear in bacterioplankton (Fig. 2B). The bacterial rates are modest: neither increasing more than 5% per year in spring, nor decreasing more than –5% per year in fall. In the Labrador Sea, the "spring-down" of chlorophyll is accompanied by a slight reduction of bacterioplankton (–2% per year) in both LS and LB. On the GS, chlorophyll and bacterioplankton have matched increases of about 7% per year (Fig. 2B).

In comparing bacterioplankton change (y) to chlorophyll change (x), the observations are found to lie exclusively in quadrant 1 ($x > 0$, $y > 0$) and quadrant 3 ($x < 0$, $y < 0$) (Fig. 4B). Thus, although bacterial change is statistically weak in individual subregions (table S1), it bears a strong correlation to chlorophyll change over a large area of the northwest Atlantic Ocean ($r = 0.83$, $n = 9$, $P = 0.006$). The reduced major axis slope of 0.42 ± 0.22 indicates that chlorophyll elicits a significantly dampened response from bacterioplankton. This is not surprising because chlorophyll has a much larger dynamic range than bacterioplankton in the ocean (*17*). It is known that marine bacterioplankton are highly diverse in phylogenetic affiliation, such that even the most common clade SAR11 (*Pelagibacter ubique*) comprises subclades or ecotypes that have different niches (*18*). Moreover, growth rates can differ greatly among phylogenetic groups such that group-specific increases can far exceed the bulk increase of the bacterial assemblage (*19*, *20*). The modest long-term bulk changes evidenced on the Scotian Shelf and Labrador Sea may be obscuring more significant change in the most active components of the microbial community.

The covariation between bacterioplankton and phytoplankton has been described as one of the few undisputed patterns in aquatic microbial ecology (*21*). The basis for this lies in the organic substrates supplied to the hetero-

trophs from the photoautotrophs. These substrates may be labile photosynthates exuded by phytoplankters, may be egesta released by grazers that have consumed phytoplankters, or may be cytoplasmic materials liberated by viral lysis or algal autolysis (*22*). The trophic groups are linked by a flux of organic matter; therefore, trophic covariation would most appropriately be sought from the carbon biomasses (mg C m^{-3}) of the two plankton groups. We have not measured carbon directly, choosing instead to represent the quantities by proxy: numerical abundance of bacterioplankton (*N*, cells m^{-3}) and chlorophyll concentration of phytoplankton (*Chl*, mg chlorophyll m^{-3}). A question inevitably arises: Are these proxies suitable to demonstrate trophic linkage, or are they inadequate because carbon biomass varies much more strongly with other factors such as cell size and pigment content, which change with physiological acclimation and taxonomic composition? On a seasonal basis, large differences may be evident in the carbon-to-chlorophyll ratio of phytoplankton, as well as in the carbon cell quota for bacterioplankton. Thus, multiyear analysis of *Chl* and *N* partitioned by seasons (Figs. 1 to 4) can be expected to be more robust because cellular parameters are more tightly constrained within seasons.

Notwithstanding seasonal differences in cellular parameters, extensive cross-ecosystem comparisons have repeatedly shown that $\log N \propto 0.46 \log Chl$ (*17*, *21*). This is a statistical trend describing the first-order relationship between the two proxies. There is much scatter of individual data about this trend, some of which can undoubtedly be explained by physiology and taxonomy. Nonetheless, the macroecological link between *N* and *Chl* through the exponent of 0.46 is consistent with the large-scale, multiyear damped response of *N* to *Chl* through the factor of 0.42 between their percentage changes (Fig. 4B).

Multiyear trends of *Chl* discerned from repeating annual cycles can be meaningfully interpreted in the context of climate variability (*14*, *23*). Trophic linkage of primary to secondary producers propagates the climate signal systemwide. Pelagic food webs in which phytoplankton are of small average cell size tend to be sustained by regenerated production, where a large fraction of energy loops through microbial components. Conversely, food webs in which phytoplankton are of large average cell size have a greater potential to transfer primary production to higher trophic levels (*24*). The ecosystem and biogeochemical implications of a long-term change in *Chl* depend on how phytoplankton biomass is packaged into discrete cells. Thus, an allometric approach (*25*) or a taxonomic approach (Fig. 1, C and D) to ecosystem monitoring yields important insights. For example, in the western subarctic North Pacific, a long-term decline in annual net community production is associated with a large springtime decrease in *Chl*, which in turn is due to a reduction in a particular subset of diatoms taxonomically identified as spring-type species (*1*).

A seasonal sign switch embedded in a multiyear phytoplankton trend has been documented elsewhere (*1*), but not, to our knowledge, a tandem sign switch in bacterioplankton. This is a compelling indication of trophic coupling at the temporal and spatial scales appropriate to climate change. The standing stock of chlorophyll is dependent on the stratification of the water column (fig. S3), implying that the observed changes are plausibly explained by changes in the delivery of deep nutrients to the surface. Within our data set, we are unable to discern a statistical correlation between the rate of change in chlorophyll versus the rates of change in temperature or the stratification index. However, much longer records of 30 to 40 years are often required to detect such linkages (*1*–*3*). Microbial observatories in the ocean directed toward long-term, spatially distributed investigations can be expected to contribute toward an understanding of ecosystem change.

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Supporting Online Material

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Dendritic Cell Apoptosis in the Maintenance of Immune Tolerance

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Apoptosis in the immune system is critical for maintaining self-tolerance and preventing autoimmunity. Nevertheless, inhibiting apoptosis in lymphocytes is not alone sufficient to break self-tolerance, suggesting the involvement of other cell types. We investigated whether apoptosis in dendritic cells (DCs) helps regulate self-tolerance by generating transgenic mice expressing the baculoviral caspase inhibitor, p35, in DCs (DC-p35). DC-p35 mice displayed defective DC apoptosis, resulting in their accumulation and, in turn, chronic lymphocyte activation and systemic autoimmune manifestations. The observation that a defect in DC apoptosis can independently lead to autoimmunity is consistent with a central role for these cells in maintaining immune self-tolerance.

The critical role of apoptosis in maintaining peripheral tolerance is clearly demonstrated by systemic autoimmune diseases that result from mutations in the pro-apoptotic Fas receptor or Fas-ligand genes, both in humans and mice (*1*–*3*). Although lymphocytes play a central role in these conditions, the extent to which apoptosis defects in various immune cell types might also be involved has yet to be fully characterized. Transgenic mice

expressing apoptosis inhibitors in T cells do not display autoimmune symptoms (*4*–*10*), and conditional deletion of Fas in T or B cells fails to induce the typical autoimmune diseases observed with global mutations in Fas (*11*). Such results make it likely that defective apoptosis in other cell types plays a prominent role in the onset of autoimmune diseases.

DCs are potent antigen-presenting cells that initiate lymphocyte activation and may also be critical for maintaining immune tolerance (*12*–*16*). Although immunization with excessive activated DCs induces tissue-specific and systemic autoimmune symptoms (*17*, *18*), the role of DC turnover in the development of autoimmunity remains untested. Previously, we observed DC accumulation in autoimmune patients harboring a deficiency in apoptosis (*19*), and significant expansion of DCs has also been reported in Fas-deficient *lpr* mice (*20*). It is plausible, therefore, that defects in DC apoptosis lead to DC accumulation, and through chronic lymphocyte activation, the development of autoimmunity (*19*).

To directly test this hypothesis, we generated independent DC-specific transgenic mouse lines (DC-p35; fig. S1) that express the baculovirus p35 protein under the control of the CD11c promoter (*21*, *22*). p35 is capable of inhibiting caspase-8 and several downstream caspases through covalent binding to active sites of these proteases (*23*). We also generated different lines of transgenic mice selectively expressing p35 in T cells (T-p35) or B cells (B-p35) under the control of the CD2 and CD19 promoters (*24*, *25*), respectively (fig. S1).

DCs, but not T or B cells, from DC-p35 mice were deficient in Fas-mediated apoptosis as compared to those cells from control mice (figs. S1 to S4). In young DC-p35 mice (1 to 3 months), no obvious changes in lymphocytes and DCs were observed (*26*). However, significant expansion of $CD11c^+$ (and $CD40^+$ or $I\text{-}A^{b+}$) DCs was observed in the spleens of DC-p35 mice but not in their nontransgenic littermates or age-matched T-p35 and B-p35 mice at 12 months of age (Fig. 1A). Expansion of $CD11c^{low}CD11b^-B220^+Gr\text{-}1^+$ plasmacytoid DCs (pDCs) (*27*) was also observed (Fig. 1B). No significant changes were detected in T cells, B cells, natural killer (NK) cells, or macrophages from DC-p35 mice (fig. S4). These data suggest that inhibition of apoptosis in DCs leads to an accumulation of DCs in DC-p35 mice with increasing age.

Marked increases in the activation marker, CD69, were detected in T and B cells in the spleens of DC-p35 mice (Fig. 1C). In contrast, only modest increases in $CD69^+$ T and B cells from T-p35 or B-p35 mice were observed (Fig. 1C), and DC-p35 mice displayed no significant change in the numbers of $CD4^+FoxP3^+$ natural T regulatory (Treg) cells relative to nontransgenic controls (Fig. 1C). Moreover, purified $CD4^+CD25^+$ cells composing the Treg population from DC-p35 and control mice inhibited the proliferation of $CD4^+CD25^-$ T cells to a similar extent (fig. S5). Negative selection and Treg development also appeared to be normal in the thymus of DC-p35 mice (fig. S6).

Because caspase-8 deficiency is known to impair the development and activation of T cells (*28*, *29*), it is possible that p35 disrupts the development of DCs in DC-p35 mice by inhibiting caspase-8. However, we found normal development of different subsets of $CD11c^+$ DCs in 1- to 3-month-old DC-p35 mice (*26*). In addition, continuous labeling of DCs with bromodeoxyuridine (BrdU) in vivo (*22*) indicated that endogenous DCs were replaced by newly generated $BrdU^+$ DCs at normal rates in DC-p35 mice (Fig. 2A), suggesting that transgenic expression of p35 was not impairing the development of DCs.

In light of these results, we examined whether the in vivo survival of DCs during an active immune response might be affected in DC-p35 mice. Mice were injected with BrdU, followed by ovalbumin (OVA) immunization (*22*). The percentage of $BrdU^+$ myeloid DCs (mDCs) in the draining lymph nodes rapidly decreased from 65% on day 1 to 20% on day 3 after immunization in wild-type mice, whereas the decrease in $BrdU^+$ mDCs was slower in DC-p35 mice (Fig. 2B). In contrast to these effects on mDCs, the number of $BrdU^+$ pDCs increased rapidly after immunization in wild-type mice (Fig. 2B). It is possible that antigen stimulation triggered proliferation of recently labeled pDC precursors, leading to a rapid expansion of pDCs during the initial phase of an immune response when the draining lymph nodes sharply increase in size. The percentage of $BrdU^+$ pDCs then decreased rapidly from 62% on day 2 to 27% on day 4 and more slowly thereafter in wild-type mice (Fig. 2B). Although the initial expansion of $BrdU^+$ pDCs was similar in DC-p35 and control mice, the decline in $BrdU^+$ pDCs was significantly slower in DC-p35 mice (Fig. 2B). These data are consistent with an increase in the survival of recently expanded mDCs and pDCs in DC-p35 mice in an immune response.

To determine whether increased survival of DCs in DC-p35 mice would lead to an increase in T cell priming in vivo, $CD8^+$ T cells from OVA-specific T cell receptor (TCR) transgenic mice (OT1) labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE) were transferred into recipient mice, followed by immunization with DCs from DC-p35 or control mice pulsed with the OVA antigen (*22*). DCs from DC-p35 mice induced stronger T cell proliferation, assayed by CFSE dilution and expansion of OT1 T cells stained with an $H\text{-}2K^b$/OVA tetramer (Fig. 2C, upper and middle panels) (*22*). Similarly, DCs from DC-p35 mice pulsed with OVA antigen induced increased proliferation of OVA-specific $CD4^+$ OT2 T cells in vivo (Fig. 2C, lower panels) and in vitro (Fig. 2D). Enhanced immune responses were also observed in DC-p35 mice by immunization with protein antigens (fig. S7). Thus, DCs from DC-p35 mice had an enhanced capacity to induce antigen-specific immune responses.

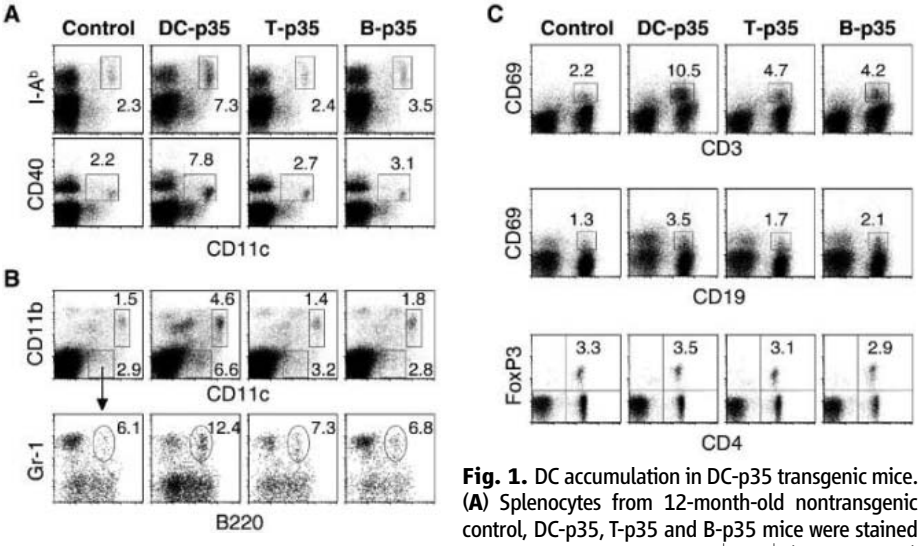

Fig. 1. DC accumulation in DC-p35 transgenic mice. (**A**) Splenocytes from 12-month-old nontransgenic control, DC-p35, T-p35 and B-p35 mice were stained for different markers and analyzed by flow cytometry. The percentage of $CD11c^+I\text{-}Ab^+$ (upper panels) or $CD11c^+CD40^+$ DCs (lower panels) is shown. (**B**) The $CD11c^{high}CD11b^+$ mDCs (upper panels) and the $Gr\text{-}1^+B220^+$ pDCs among the $CD11c^{low}CD11b^-$ cells (lower panels) were stained and analyzed by flow cytometry. (**C**) CD69 expression on $CD3^+$ cells (upper panels) or $CD19^+$ cells (middle panels) was analyzed by flow cytometry. Splenocytes were also stained with fluorescein isothiocyanate (FITC)–anti-CD4 (antibody to CD4), followed by intracellular staining with phycoerythrin (PE)–anti-FoxP3 (lower panels).

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In adoptive-transfer experiments, DCs from DC-p35 or *lpr* mice were found to be potent inducers of antinuclear antibodies (ANAs) in recipient mice (Fig. 2E). Bcl-2 have also been shown to prolong the survival and enhance the immunogenicity of DCs (*30*). However, compared with p35-transduced DCs, bcl-2–transduced DCs were less efficient at inducing ANAs (Fig. 2E; fig. S7F). Thus, in addition to inducing enhanced antigen-specific immune responses, p35-DCs have the potential to induce autoantibody production.

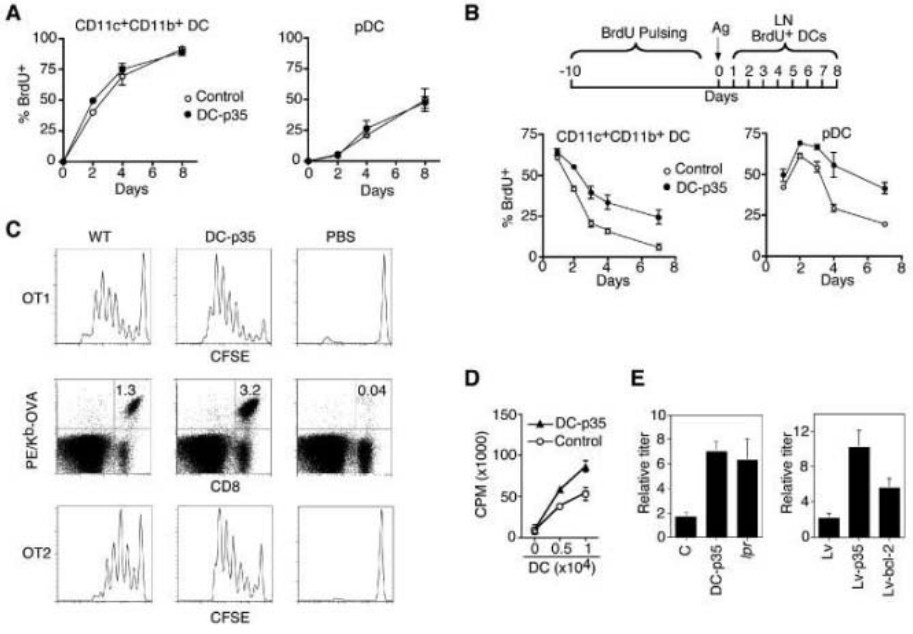

Fig. 2. Enhanced immune responses in DC-p35 mice. (**A**) Percentage of $BrdU^+$ DCs in the spleens of DC-p35 or nontransgenic mice after continuous BrdU labeling (*22*). (**B**) DC-p35 or nontransgenic mice were pulsed with BrdU from day −10 to day −1 and immunized with OVA on day 0. The percentage of $BrdU^+$ cells among mDCs or pDCs in draining lymph nodes (LN) was quantitated at different times after immunization. (**C**) CFSE-labeled OVA-specific TCR transgenic $CD8^+$ OT1 (upper panels) or $CD4^+$ OT2 T cells (lower panels) and DCs from DC-p35 or control mice pulsed with the corresponding OVA peptides were transferred into recipient mice (*22*). $CFSE^+$ cells in the draining nodes were gated to show CFSE dilution of dividing cells. Expansion of $CD8^+$ OVA-specific T cells was also probed with H-$2k^b$/OVA-tetramer (middle panels). Phosphate-buffered saline (PBS) solution was used instead of DCs for negative controls. (**D**) $CD4^+$ T cells (10^5 per well) from OT2 mice were mixed with the indicated numbers of DCs of DC-p35 mice and controls in the presence of OVA (10 μg/ml) for 3 days. Cell proliferation was measured by thymidine uptake. (**E**) DCs from DC-p35, *lpr*, or control (C) mice (left) or DCs from C57BL/6 mice transduced with a lentiviral vector (Lv), Lv-p35, or Lv–bcl-2 (right) were transferred into recipients. After 24 hours, recipients were injected with lipopolysaccharide, and sera were collected 2 weeks later to quantitate ANAs (*22*). Data (mean ± SD) were analyzed by Student's *t* test using GraphPad Prism version 4 for Macintosh. A *P* value of <0.05 was considered statistically significant.

To further investigate the potential for increased DC survival in inducing autoimmunity, we first compared spontaneous production of autoantibodies in both young and old DC-p35 mice. We detected no increases of ANAs in DC-p35 mice at 3 and 6 months of age (fig. S8). However, ANAs could be detected in 9-month-old DC-p35 mice (fig. S8). By 12 months of age in DC-p35 mice, ANAs could be found in most DC-p35 mice, but not in nontransgenic littermates or age-matched T-p35 and B-p35 mice (Fig. 3A). Significant ANA production was also detected in the independent DC-p35 transgenic line 2 (Fig. 3A), suggesting that autoantibody production was not due to a positional effect of the transgene. Unexpectedly, crossing DC-p35 to T-p35 mice (DC/T-p35) or B-p35 mice (DC/B-p35) did not significantly affect the titers of ANAs (Fig. 3A), suggesting that additional inhibition of apoptosis by p35 in T or B cells was not an important factor in promoting autoantibody production in DC-p35 mice. Substantial DC accumulation and lymphocyte infiltration in the lung were evident in all 12-month-old DC-p35 mice with detectable ANAs, but not in T-p35 or B-p35 mice (Fig. 3B). Most 12-month-old DC-p35 mice (>80%) also displayed immunogloblin G (IgG) deposition in the glomeruli of kidneys (Fig. 3B), whereas lymphocyte infiltration in the livers and kidneys was detectable in only ~20% of 12-month-old DC-p35 mice (*26*). Our data indicate that DC-p35 mice on the C57BL/6 background developed autoimmune manifestations at old ages.

A four-generation backcross of DC-p35 mice to the autoimmune-prone *MRL* background induced significant DC accumulation at 6 months (Fig. 4A), and greater numbers of $CD11c^+$ DCs were found outside the B cell areas and in T cell areas in the spleens of *MRL*/DC-p35 mice (Fig. 4F). An increase in spontaneous activation of T cells was also observed in 6-month-old

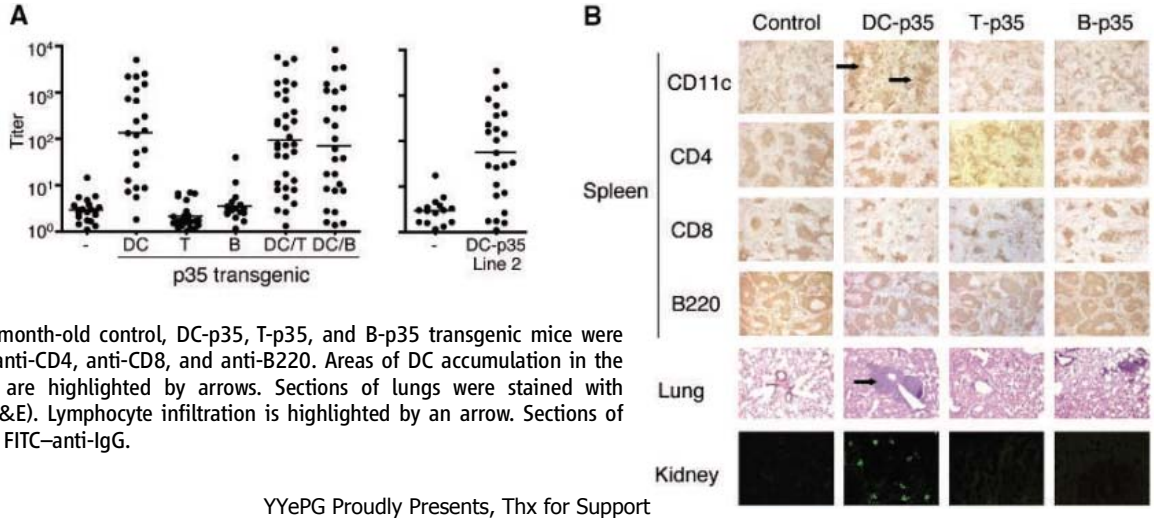

Fig. 3. Autoimmunity in DC-p35 mice on the C57BL/6 background. (**A**) Enzyme-linked immunosorbent assay (ELISA) for ANAs in the sera of 12-month-old control (–), DC-p35, T-p35, B-p35, and DC/T-p35 (left) and DC/B-p35 transgenic mice on the C57BL/6 background (right). (**B**) Sections of spleens from 12-month-old control, DC-p35, T-p35, and B-p35 transgenic mice were stained with anti-CD11c, anti-CD4, anti-CD8, and anti-B220. Areas of DC accumulation in the spleens of DC-p35 mice are highlighted by arrows. Sections of lungs were stained with hematoxylin and eosin (H&E). Lymphocyte infiltration is highlighted by an arrow. Sections of kidneys were stained with FITC–anti-IgG.

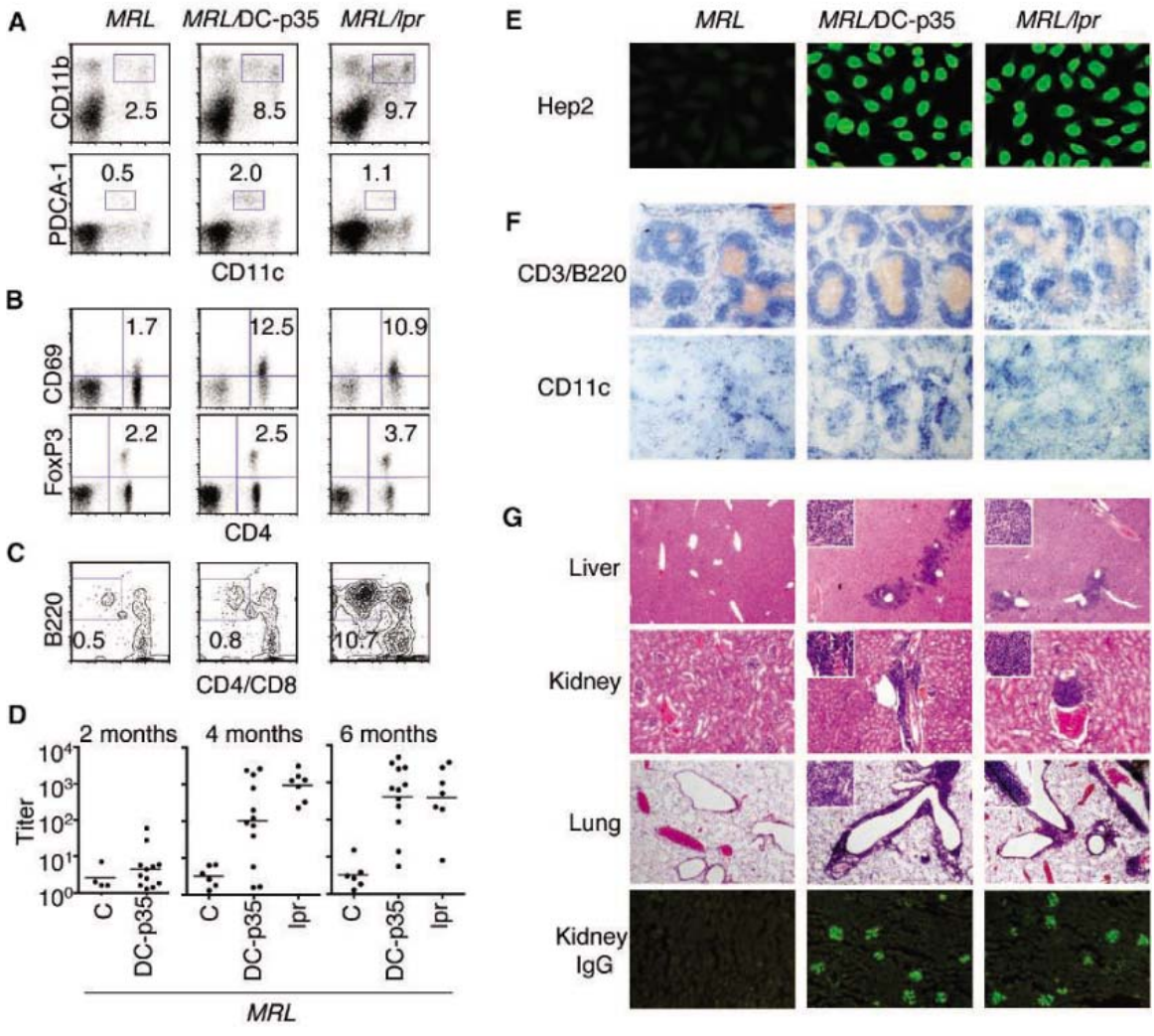

Fig. 4. DC accumulation, lymphocyte activation, and autoimmunity in DC-p35 mice on the *MRL* background. (**A** to **C**) Total splenocytes from 6-month-old DC-p35 mice, nontransgenic control, and *lpr* mice on the *MRL* background were stained for $CD11c^+CD11b^+$ mDCs (A) (upper panels) or plasmacytoid dendritic cell antigen–1 (PDCA-1)–positive and $CD11c^{low}$ pDCs (A) (lower panels), CD69 on T cells (B) (upper panels) or with FITC–anti-CD4 followed by intracellular staining with PE–anti-FoxP3 (B) (lower panels), or with FITC–anti-TCR$\alpha\beta$, PE–anti-CD4, PE–anti-CD8, and cychrome–anti-B220 (C). $TCR\alpha\beta^+$ cells were gated and $B220^+CD4^-CD8^-$ DNT cells were quantitated (C). (**D**) ANA titers in the sera of control (C), DC-p35, and *lpr* mice on the *MRL* background were determined by ELISA. (**E**) ANAs were also detected by incubating sera from 6-month-old *MRL* (1:40 dilution), *MRL*/DC-p35 (1:640), and *MRL/lpr* (1:640) mice with Hep2 cell slides followed by probing with FITC-conjugated anti-mouse IgG. (**F**) The spleen sections of 6-month-old control, DC-p35, and *lpr* mice on the *MRL* background were stained for T cells (anti-CD3, red) and B cells (anti-B220, blue) (upper panels) or DCs (anti-CD11c, blue) (lower panels) (*22*). (**G**) H&E staining of lungs, livers, and kidneys of 6-month-old mice on the *MRL* background (*22*). The kidney sections were also stained with FITC–anti-mouse IgG.

MRL/DC-p35 mice (Fig. 4B) (*26*), although again, normal numbers of $CD4^+FoxP3^+$ Treg cells were present (Fig. 4B). DC accumulation and chronic lymphocyte activation were similar in DC-p35 and *lpr* mice, but *MRL*/DC-p35 mice did not show expansion of the unusual $TCR\alpha\beta^+B220^+CD4^-CD8^-$ double-negative T (DNT) cells that are abundant in *MRL*/*lpr* mice (Fig. 4C), suggesting that the accumulation of DNT cells is not caused by apoptosis deficiency in DCs. *MRL*/DC-p35 developed significant levels of ANAs at 4 months of age (Fig. 4D), and by 6 months of age, most *MRL*/DC-p35 mice had developed ANAs (Fig. 4D). Sera from these DC-p35 mice showed speckled nuclear staining of Hep2 cells similar to that of the *lpr* sera (Fig. 4E). Lymphocyte infiltrations near the bronchi in the lungs and in the liver and kidney surrounding the blood vessels, and IgG deposition in the glomeruli of kidneys, were observed in all 6-month-old *MRL*/DC-p35 and *MRL-lpr* mice with detectable ANAs (Fig. 4G). Therefore, DC-p35 mice have an earlier onset of autoimmunity on the *MRL* background.

Although lymphocytes are likely to be essential in inflicting autoimmune damage, our finding that apoptosis in DCs can independently lead to autoimmune manifestations suggests that these cells may be key initiators of autoimmune responses in individuals harboring apoptosis deficiencies. Negative selection and the development of Treg cells in the thymus were apparently normal in DC-p35 mice (fig. S6), suggesting that only peripheral tolerance is affected. DC accumulation due to apoptosis deficiency in DC-p35 mice may selectively induce overactivation of responder lymphocytes, resulting in the onset of systemic autoimmunity. On the autoimmune-prone *MRL* background, DC-p35 mice developed accelerated autoimmune responses (Fig. 4), indicating that synergies between apoptosis deficiency in DCs and other genetic and environmental factors likely will induce more severe autoimmune symptoms. The critical role of DC apoptosis in regulating peripheral tolerance suggests that targeting DCs may represent an effective therapeutic approach to limiting the onset of autoimmune diseases.

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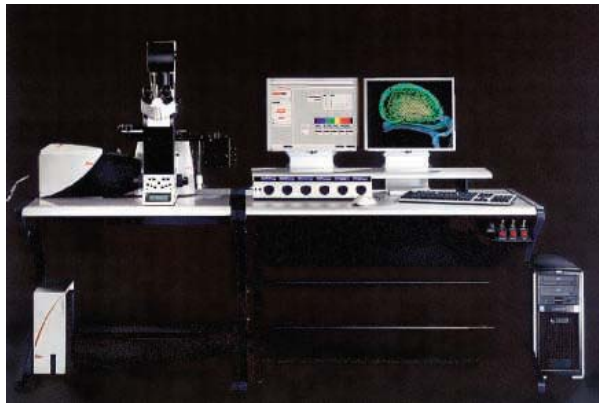

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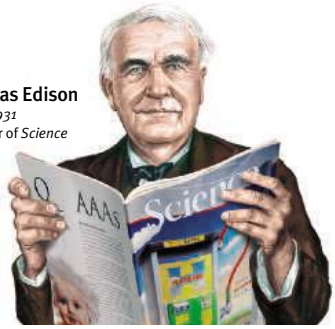

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POSITIONS OPEN

FACULTY POSITION
Department of Molecular Biology
Princeton University

The Department of Molecular Biology at Princeton University invites applications for a tenure-track faculty position at the Assistant Professor level. We are seeking an outstanding investigator in proteomics with special emphasis on mass spectrometry. Ph.D.s or M.D.s with postdoctoral research experience should send curriculum vitae, short summary of research interests, and three letters of reference to: **Proteomics Search Committee, c/o Gail Huber, Department of Molecular Biology, Princeton University, Princeton, NJ 08544-1014.** For full consideration applications should be received by April 1, 2006. For additional information about the Department, visit our **website: http://www.molbio.princeton.edu.** For information about applying to Princeton and how to self-identify, please link to Applicant Self-Identification Form.

Princeton University is an Equal Opportunity/Affirmative Action Employer.

PHYSICIAN-SCIENTIST: ASSISTANT/ ASSOCIATE/ PROFESSOR

The Veterans' Administration Palo Alto Health Care System and Stanford University School of Medicine are seeking highly qualified applicants for a position of Assistant, Associate or Full Professor in the Department of Medicine. Applicants should be Physician-Scientists with interests in basic and translational research in an area such as cancer biology, cardiovascular or pulmonary science, microbiology and host defense, immunology, developmental biology, or genetics/genomics. Applicants should have either an M.D. or M.D./Ph.D., or equivalent degrees, and should have a strong record of accomplishments and a track record of obtaining extramural research funding. Board certification in internal medicine and in a medical subspecialty is required. The appointee will be expected to conduct research in a collaborative fashion and to play a leadership role within the appropriate Division and research program at Stanford University.

Applicants should send their curriculum vitae and the names, addresses, telephone numbers, and e-mail addresses of three references to:

Attention: Fredric B. Kraemer, M.D.
ACOS, Research and Development (151A)
VA Palo Alto Health Care System
3801 Miranda Avenue
Palo Alto, CA 94304

Stanford University is an Equal Opportunity Employer and is committed to increasing the diversity of its faculty. It welcomes nominations of and applications from women and members of minority groups, as well as others who would bring additional dimensions to the University's research, teaching and clinical missions.

The University of Arizona, College of Medicine invites applications for **HEAD, DEPARTMENT OF IMMUNOBIOLOGY.** The University of Arizona College of Medicine invites applications and nominations for a position that entails a unique opportunity to serve as Head and work with the Dean and the faculty to formulate the direction for a new Department of Immunobiology. The competitive recruiting package includes an attractive salary, generous laboratory space, and resources for new faculty hires, equipment, personnel and operating expenses.

Details are available at **website: http://medicine.arizona.edu/search** or direct inquiries to **Dr. Marilyn Halonen** at: **e-mail: mhalonen@e-mail.arizona.edu.** Review will begin April 1, 2006. *The University of Arizona is an Equal Employment Opportunity/Affirmative Action, Minorities/Women/Persons with Disabilities/Veterans Employer.*

POSITIONS OPEN

DEVELOPMENTAL NEUROSCIENCE

The Department of Anatomy at the University of Wisconsin (UW), Madison invites applications for a tenure-track position at the rank of **ASSISTANT PROFESSOR** effective August 1, 2006. We seek applicants with a strong research program in the area of Developmental Neuroscience with a focus on the molecular basis of axon growth and pathfinding. Candidates using genetic model systems such as mouse, zebrafish, and drosophila are particularly encouraged to apply as work in this area will complement existing strengths in our Department. (Current faculty research interests at UW, Madison can be found at **website: http://www.anatomy.wisc.edu/research.html.**) Possible areas of specialization include: the regulation of cell differentiation, axon growth and guidance by intracellular signals, molecular basis of cytoskeletal rearrangements during neuronal migration and growth cone motility, development of high resolution, and high throughput imaging approaches to study the cellular and physiological basis of cell motility. Candidates will be expected to show evidence of excellence in both research and teaching. A Ph.D., M.D., or M.D./Ph.D. and two or more years of postdoctoral research are required. The successful applicant will conduct an active fundable research program, participate in training Ph.D. candidates and postdoctoral fellows, participate in teaching one of the courses in the Department, and participate in University and public service. Applicants should submit detailed curriculum vitae, a statement of research interests and goals, representative publications, and the names and addresses of three references to: **John K. Harting, Ph.D., Professor and Chair, University of Wisconsin Department of Anatomy, 1300 University Avenue, Madison, WI 53706-1532.** To ensure consideration, applicants must submit application materials on or before April 3, 2006.

Note: Unless confidentiality is requested in writing, information regarding the names of applicants must be released upon request. Finalists cannot be guaranteed confidentiality.

UW, Madison is an Equal Opportunity/Affirmative Action Employer. We promote excellence through diversity and encourage all qualified individuals to apply.

FACULTY POSITIONS (Three)
CHEMISTRY (Two), MICROBIOLOGY
Azusa Pacific University

Chemistry, specialty open. Candidates with doctorate in chemistry are sought to teach major and nonmajor chemistry lecture and laboratory courses within candidate's expertise.

Microbiology. Candidates with doctorate in microbiology or a related field are sought to teach lecture and laboratory courses in microbiology and other courses within candidate's expertise.

Additional Qualifications: Commitment to teaching excellence in an undergraduate liberal arts program, a record of scholarship and willingness to conduct research in an undergraduate setting, and a vibrant Christian faith compatible with the University "Statement of Faith." Interested applicants should download an application from **website: http://www.apu.edu/provost/facultyEmployment/.** Additional materials may be requested from selected applicants. Position is open until filled. Funding pending. *Women and minorities are encouraged to apply.*

MICROBIOLOGY POSITION

Tenure-track **ASSISTANT PROFESSOR** position. Introductory microbiology is the primary teaching assignment. Develop other courses and research program in areas of expertise. Preference will be given to those able to develop introductory courses in immunology and toxicology. Position open until filled. Candidates should send resume and the names of three references to: **Chair, Microbiology Search Committee, Department of Biology, c/o Human Resources, P.O. Box 11127, Lamar University, Beaumont, TX 77710.**

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Faculty Positions

A Tale of Two Systems: Tenure v. Adjunct

Colleges and universities hire faculty as adjunct or tenure-track. The adjunct approach provides desirable flexibility for the employer and, sometimes, for the employee, too. Still, most academics seek tenure-track positions. The experts interviewed here discuss the pros and cons of tenure and adjunct systems. BY MIKE MAY

Brown University
http://www.brown.edu/

The University of British Columbia
http://www.ubc.ca/

Whitman College
http://www.whitman.edu

Almost every student pursuing a career in academics desires the same ultimate goal: a tenure-track position. This achievement means essentially a life-time position, intellectual freedom, and a sort of status in the field. Nonetheless, searches to fill tenure-track positions grow continually more competitive, as the number of jobs dwindles. According to "Staff in Postsecondary Institutions, Fall 2003, and Salaries of Full-Time Instructional Faculty, 2003-04," which was issued by the U.S. Department of Education, the total number of postsecondary faculty grew by 26 percent from 1995 through 2003, but the number of full-time faculty on the tenure track increased by only 17 percent. During the same period, the number of part-time faculty increased by 43 percent. Consequently, anyone seeking a tenure-track position faces a significant challenge. Still, the experts interviewed here see strength in the tenure system and great career options now and in the future.

In essence, the divide lies between tenure-track and adjunct positions. Patrick Keef, dean of the faculty and professor of mathematics at Whitman College in Walla Walla, Washington, clearly draws the distinction. He says, "A tenure-line position is a permanent commitment by a college, presuming that a faculty member meets the standards of excellence for the duration of a career." He adds, "An adjunct position is temporary in time and may be unstable in the amount of teaching classes given to the individual."

An Adjunct Overview

Although tenure-track is the most common goal, adjunct positions offer benefits too. Rajiv Vohra, dean of the faculty and professor of economics at Brown University, says, "An adjunct position is often a good way for people with an interest in teaching related to their profession al jobs to teach without giving up their main careers." Keef adds that "some employees are interested in the flexibility of being an adjunct. They may not be interested in the pressures of a full-time position."

In addition, Vohra points out that adjunct positions benefit an institution. He says, "There are areas in which demand may be more than academic. It could be a professional interest, such as accounting." Vohra also states that adjunct positions let institutions balance faculty needs with student interests. He says, "If the popularity of some fields changes over time, it is easier for an institution to hire adjuncts in areas of new interest. It provides a university with financial flexibility to manage things." Keef agrees, saying, "Most institutions use adjuncts for flexibility." CONTINUED »

Faculty Positions


MICHAEL HAYDEN

For example, adjunct faculty members might fill in when tenured members take sabbaticals.

Institutions might also use adjunct positions to keep a tenure-track employee. Keef says, "With a dual-career couple, where one is in a tenured position, the other might be able to contribute in a part-time capacity. That might assist the couple with a better income." He adds, "That happens a lot."

Despite the benefits of flexibility, adjunct positions do not always create a desirable situation. "Sometimes, people have part-time positions because of the reality of the marketplace, not because they want this kind of position," Vohra says. "The job market could be very tight. Some people even have several adjunct positions because that is the only way to put together a full-time salary." He concludes: "That is not something a faculty member would take as a first choice. It leads to a situation that can be difficult as a long-term plan."

Employers, for the most part, avoid too many adjunct positions. Vohra says, "The more positions that are part time or short term, the less loyalty there is toward the institution. That has a negative impact on the institution." In addition, he says, "It's difficult to specify exactly what it is that you are contracting for, in the sense that the responsibilities of a faculty member go beyond what transpires in the regularly scheduled classrooms. One is looking for a commitment that involves advising, helping students with research opportunities, and everything that happens outside the classroom."


PATRICK KEEF

Tenure: Higher Dedication

For faculty members, getting tenure offers many benefits. For one thing, tenured faculty play fundamental roles in developing the future of a department, according to Michael Hayden, director and senior scientist at the Centre for Molecular Medicine and Therapeutics and professor in the Department of Medical Genetics at The University of British Columbia. He says, "Tenured faculty members play a big role in recruitment and voice their opinions. They have a much bigger stake all around."

Keef adds that tenure includes much greater expectations for scholarship than do adjunct positions. In addition, he says, "Tenure positions are better paid, provide better benefits, and better job security." He adds, "Tenure is the gold standard." Vohra adds, "For all practical purposes, tenure is a permanent job." But he continues: "Also, the university makes tenure decisions with a long-term view, so that is the reason for the elaborate procedures to grant it."

Visit **www.sciencecareers.org** and plan to attend upcoming meetings and job fairs that will help further your career.

The tenure system, though, might not always benefit an institution. "In hiring a tenure-track faculty member," says Vohra, "an institution makes a long-term commitment, but circumstances—like the needs of the institution—might change over time. So this system comes with some baggage, to a degree." Still he adds, "The benefits turn out to be significant enough that institutions accept the idea of tenure. Anyway, the most sought-after researchers today would not accept a position that did not come with tenure."


RAJIV VOHRA

Modern Modifications

Some positions land a bit between adjunct and tenure. For example, Hayden points out that departments at The University of British Columbia often include associate members. He says, "These are people with some relation to a department, but not active members. These faculty have a primary focus outside the department and either collaborate or work with someone in the department where there is a mutual benefit." In general, though, such associate members are usually tenured in another department or center. Hayden says that this sort of connection between departments and centers improves opportunities for collaborations. He adds, "It gives strength to a department. It adds credibility."

For any sort of position, people on the job market wonder what opportunities exist. The experts interviewed here point out open positions. Hayden says, "We are recruiting three new faculty to our Centre for Molecular Medicine and Therapeutics." He adds that this search will involve candidates from around the world. Currently, this center employs 150 people from approximately 35 countries. Likewise, Vohra says, "We are going through a fairly significant expansion, hiring 100 faculty positions, and those are tenure-track positions."

The figures from the U.S. Department of Education, however, indicate a decrease in tenure-track positions over the past few years. Nonetheless, the experts interviewed here do not see a significant increase in the use of adjuncts at their institutions. Vohra says that Brown University relies mostly on tenured faculty. He says, "We have some adjuncts and some visiting professors, but there is no sense in which we have tried to increase reliance on adjuncts. Most of our faculty have tenure-track positions. We have some lecturers, but they are a small fraction of the faculty."

Although Keef concedes that the tenure system might not be as healthy as it was 30 or 40 years ago, he still sees it as a very strong system. He says, "It's my impression that people have been predicting the demise of tenure since I have been in academics, but it seems to be doing just fine."

Mike May (mikemay@mindspring.com) is a publishing consultant for science and technology based in Minnesota.

UT-ORNL
Governor's Chairs

The University of Tennessee in partnership with Oak Ridge National Laboratory is recruiting leading scientists to conduct research in the new Joint Institute of Biological Sciences with access to some of the most advanced scientific tools available. In addition to working in an exciting atmosphere of intellectual and academic freedom, you would be living in one of the most beautiful areas in the country with easy access to miles of inland waterways, pristine state and national parks, diverse cultural opportunities and a unique mix of convenient urban and rural living settings.

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Governor's Chairs in the UT-ORNL Joint Institute of Biological Science

The State of Tennessee is investing funds to recruit and support approximately 20 exceptionally accomplished researchers who will have joint appointments as tenured professors at the University of Tennessee (UT) and distinguished research staff at the Oak Ridge National Laboratory (ORNL). This Governor's Chair (GC) program seeks to catalyze the development of cutting edge research under the auspices of four joint institutes between the UT and the ORNL: Biological Sciences, Computational Sciences, Neutron Sciences, and Advanced Materials Sciences. The GC appointments include an ongoing discretionary research fund equal to twelve months salary.

The Joint Institute for Biological Sciences (JIBS)
JIBS will support research and teaching programs in genomics, bioinformatics and computational biology, molecular structural biology, proteomics, and biomedical technologies. JIBS will encompass both fundamental and applied research and development across a spectrum of systems, from microbial to mammalian, accelerating translation of insights into novel biotechnologies and theorems. Included in JIBS is the Genome Science and Technology Graduate School offered jointly by UT and ORNL.

The UT-ORNL environment nurtures a rich interdisciplinary community of researchers with common interests and collaborative projects. The UT-ORNL research enterprise also has more than $2 billion in investments in some of the world's most advanced research facilities.

There are immediate openings for Governor's Chairs in the following areas:

- **Microbiology**, microbial physiology and genomics, understanding the dynamics of microbial communities, with emphasis on those microbial systems relevant to applications in bio-energy and in ecosystem dynamics.
- **Plant Biology**, plant genomics, genetics and biochemistry of photosynthesis, disease resistance, plant-microbe interactions, biomass production.
- **Molecular Biophysics**, cellular and molecular imaging, nano-bio interfaces and molecular machines, understanding and predicting protein structure and dynamics and macromolecular interactions.
- **Mouse Genetics and Genomics**, the mouse as a model organism for a wide variety of physiological and developmental processes, with an emphasis on study and genetic dissection of complex traits.

UT and ORNL have strong research efforts in these areas. The research environment favors cross-disciplinary cutting-edge efforts that leverage special facilities in the physical and computational sciences. World-class research facilities include a modern SPF rodent facility with capacity of up to 70,000 animals, leading microbial genomics capabilities, state-of-the-art plant biology facilities, leading biological mass spectrometry capabilities, world-class bioinformatics and computational biology, high-performance computing systems, and the Spallation Neutron Source, a $1.4-billion federally-funded user facility on schedule for completion in 2006, that will provide the world's most intense neutron beams, among others, for a number of applications in biology.

Successful candidates will have an exceptional record of scientific productivity and accomplishment, as manifested, for example, in high-impact publications, scientific awards, or Fellow status in scientific and engineering societies. Successful candidates will also have a demonstrated record of leading cross-disciplinary teams of researchers, and of developing substantial externally-funded research programs.

APPLICATIONS: Applicants should submit a letter of interest and a curriculum vita to : Dr. Jeffrey M. Becker, Chair, JIBS-Governors Chair Search Committee, University of Tennessee, Department of Microbiology, M409 Walters Building, Knoxville, TN 37996-0845; jbecker@utk.edu. Screening of applications will commence on March 1, 2006, and will continue until the positions are filled. The University of Tennessee is an EEO/AA/Title VI/Title IX/Section 504/ADA/ADEA institution in the provision of its education and employment programs and services.

Scientists and engineers at Oak Ridge National Laboratory (ORNL) and the University of Tennessee (UT) conduct basic and applied research and development to create scientific knowledge and technological solutions that strengthen the nation's leadership in key areas of science; increase the availability of clean, abundant energy; restore and protect the environment; and contribute to national security. UT and ORNL provide an environment that encourages collaborative research and development. UT-Battelle manages and operates ORNL.

UT-BATTELLE

THE UNIVERSITY of TENNESSEE

OAK RIDGE National Laboratory

SCOTT & WHITE

College of Medicine
The Texas A&M University System Health Science Center

Pediatric Hematology-Oncologist

The Section of Pediatric Hematology/Oncology at **Scott and White Clinic** and the **Texas A&M University System Health Science Center College of Medicine** (TAMUS HSC-COM) are seeking a clinician scientist with current research grants for a faculty position in a rapidly growing program. The candidate should be BE/BC in pediatric oncology and committed to an academic career. The successful candidates will join and enhance ongoing efforts in basic and translational research, with an institutional commitment to building a world-class experimental therapeutics program. An outstanding start-up package includes high quality laboratory space, excellent benefits and competitive salaries commensurate with academic qualifications. The position guarantees 75% protected time for research activities.

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Please contact: **Don Wilson, M.D. Professor and Chairman, Department of Pediatrics, Scott & White, 2401 S. 31st, Temple, TX 76508. (800)725-3627 dwilson@swmail.sw.org Fax (254) 724-4974.**

For more information about Scott & White, please visit www.sw.org For Texas A&M www.tamhsc.edu. Scott & White is an equal opportunity employer.

UNIVERSITY OF ROCHESTER MEDICAL CENTER

The University of Rochester is seeking:

Faculty Member – Immunology

The Center for Vaccine Biology and Immunology in the University of Rochester Medical Center is recruiting a new Faculty Member at the Assistant, Associate or Full Professor level. Laboratory space will be available in the Aab Institute for Biomedical Research, adjacent to other Center Faculty. An academic appointment at the appropriate rank will be offered in the Department of Microbiology and Immunology. We seek an energetic scientist whose research complements our own, and opens up the possibility of synergy within the Center and the wider Medical Center community. We are particularly interested in researchers who are investigating basic immunobiology in human systems, studying innate immunity, or using modern imaging technologies, but these criteria do not overshadow excellence and we invite all qualified investigators to apply.

Please send your CV, a 1-2 page summary of your research interests, and the names of and contact information for three referees to:

Professor Ian N Crispe
Chair, Search Committee
Associate Director
David H Smith Center for Vaccine Biology and Immunology
University of Rochester
601 Elmwood Avenue
Rochester, NY 14642, USA

All applications will be held in confidence.

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VANDERBILT UNIVERSITY
SCHOOL OF MEDICINE

FACULTY POSITION IN VIROLOGY
Department of Microbiology and Immunology

The Department of Microbiology and Immunology at Vanderbilt University School of Medicine invites applications for a tenured or tenure-track faculty position in virology at the Associate Professor or Assistant Professor level. We are seeking an individual with an outstanding record of accomplishments in the study of DNA viruses to complement the department's existing strengths in RNA viruses and retroviruses. For information about the research activities of faculty in the department, please visit our website at **http://www.mc.vanderbilt.edu/microbio/**.

Applicants should send a curriculum vitae, a statement of current and future research interests, and three letters of recommendation to: **Dr. Christopher Aiken, Chair of Virology Search Committee, Department of Microbiology and Immunology, Vanderbilt University School of Medicine, Room A-5301, Medical Center North, 1161 21st Ave. S., Nashville, TN 37232-2363.** Inquiries, applications, and recommendation letters can be directed via email to **chris.aiken@vanderbilt.edu**. Review of applications will commence **March 1, 2006**. The deadline for receipt of applications is **May 1, 2006**.

Vanderbilt University is an Affirmative Action/ Equal Opportunity Employer.
Women and minority candidates are encouraged to apply.

HEAD of DEPARTMENT
Department of Physics
University of Illinois at Urbana-Champaign

The Department of Physics at the University of Illinois is seeking qualified applicants for the position of Head of the Department. The Department has a strong research and teaching program covering a broad range of experimental and theoretical physics. Applicants must have a Ph.D., an international reputation in research, and a strong interest in developing the research and teaching programs of the Department. The Head of the Department is the chief administrative officer with responsibilities for leading the faculty in the development of research, teaching and public service, and for administrative, budgetary and promotion decisions. The Department Head holds the rank of Professor of Physics with tenure and reports to the Dean of the College of Engineering. The proposed date of appointment is August, 2006 or as soon as possible thereafter. Salary is negotiable.

Qualified applicants should submit resumes and names and addresses of at least three references to:

Chair, Search Committee, Physics Dept Hd
University of Illinois at Urbana-Champaign,
Attn: Kathy Darr, College of Engineering
306 Engineering Hall, MC 266
1308 W. Green Street
Urbana, IL 61801
Phone: (217) 333-2151
Fax: (217) 244-7705
Email: kdarr@uiuc.edu

To ensure full consideration, resumes must be received by: **April 3, 2006**.

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FACULTY POSITIONS IN ANALYTICAL AND PARALLEL CHEMISTRY

St. Jude Children's Research Hospital
ALSAC • Danny Thomas, Founder
Finding cures. Saving children.

The Department of Chemical Biology and Therapeutics at **St. Jude Children's Research Hospital** invites applications for two faculty positions at the level of ASSOCIATE MEMBER or MEMBER. We are specifically seeking applicants currently leading established research programs in analytical chemistry and parallel chemistry for the discovery of novel bioactive small molecules.

Analytical Chemistry

The successful applicant will have a demonstrated track record in application of analytical chemistry to organic chemistry, medicinal chemistry, pharmacokinetics, or drug discovery. Appointees will establish an independent research program in analytical chemistry and direct analytical efforts for local multidisciplinary projects in the areas of chemical biology and drug discovery.

Parallel Chemistry

The successful applicant will have a demonstrated track record in the synthesis of large high-quality discovery libraries or lead optimization libraries in a medicinal chemistry or chemical biology setting. Appointees will establish an independent research program in parallel chemistry and direct diversity library production efforts for local multidisciplinary projects in the areas of chemical biology and drug discovery.

The Department of Chemical Biology and Therapeutics is one of 15 academic departments at St. Jude Children's Research Hospital. The institute encourages translational research, and has outstanding shared laboratory and clinical resources that facilitate collaborations among a highly collegial group of scientists. Extensive opportunities exist for collaboration with both clinically based and basic research programs relevant to oncology and infectious disease.

Appointees will lead a strong program in a multidisciplinary, thematically integrated Department focused on the discovery and development of small molecules for perturbing cellular functions - particularly in systems relevant to pediatric oncology and infectious disease. Individuals will contribute to one or more existing and new programs at the institution, including the interdisciplinary research programs of Developmental Therapeutics for Solid Malignancies, Hematological Malignancies, Infection & Host Defense, Molecular Oncology, Neurobiology & Brain Tumor, Signal Transduction, Transplantation & Gene Therapy, Chemical Biology, or Cancer Prevention & Control.

St. Jude offers a very competitive package for these positions, including a generous startup allowance with newly remodeled space and equipment; laboratory resources (as needed); and support positions. In addition, appointees have access to a range of institutional core facilities including protein and nucleic acid chemistry, microarray analysis, gene knockout and transgenic technologies, pharmacokinetics, and development of animal models.

Those interested in joining this multidisciplinary department should arrange to have their CV, a brief prospectus of research interests, and three letters of recommendation sent to:

R. Kip Guy, Ph.D., Chair
Department of Chemical Biology and Therapeutics
St. Jude Children's Research Hospital
322 North Lauderdale Street
Memphis, TN 38105

www.stjude.org

An Equal Opportunity Employer

TWO TENURE TRACK FACULTY POSITIONS IN IMMUNOLOGY AND INFECTIOUS DISEASES

The Division of Basic Medical Sciences, Faculty of Medicine, Memorial University of Newfoundland invites applications for two tenure track faculty positions (**VPA#2004-005** and **VPA#2005-003**) in Immunology and Infectious Diseases at the Assistant Professor level—one of these positions is expected to be in Viral Hepatitis. Candidates should possess a Ph.D. or equivalent degree, a minimum of two years of post-doctoral experience and a proven track record in the areas of immunology and infectious diseases. Couples are encouraged to apply. The successful applicants will be expected to establish independent research programs and contribute to undergraduate medical and graduate student teaching. Active areas of research within the Immunology program at Memorial University include autoimmunity, viral immunology, tumor immunology and innate immunity. Applicants may refer to the Immunology program website at **www.med.mun.ca/basic/pages/programs_immunology.htm** for more information. The Division of Basic Medical Sciences also includes active research groups and graduate programs in Neuroscience, Cancer Research and Cardiovascular/Renal Physiology. Consideration of applications will begin **April 17, 2006** and continue until the position is filled. Applicants must submit a curriculum vitae, a summary of proposed research, and provide the names and addresses of three referees. Forward to:

Dr. Karen M. Mearow (jblundon@mun.ca)
Associate Dean
Division of Basic Medical Sciences
Faculty of Medicine
Health Sciences Centre
Memorial University of Newfoundland
St. John's, Newfoundland and Labrador
A1B 3V6

Memorial University is the largest university in Atlantic Canada and, as the only university in the province, Memorial plays an integral role in the education and cultural life of Newfoundland and Labrador. The university is situated in St. John's, a very safe, friendly city with great historic charm, a vibrant cultural life, and easy access to a wide range of outdoor activities.

Memorial University is committed to employment equity and encourages applications from qualified women and men, visible minorities, aboriginal people and persons with disabilities. All qualified candidates are encouraged to apply; however, Canadian citizens and permanent residents will be given priority.

Head of Department and Professor Bioengineering
University of Illinois at Urbana-Champaign

The University of Illinois at Urbana-Champaign invites applications and nominations for the position of Head, Department of Bioengineering. The Department was established in 2003. It has undergraduate (BS) and graduate (MS, PhD) educational and research programs in bioimaging, cellular and tissue engineering, computation bioengineering, and nano- and microsystems technology. The department currently has seven full-time faculty members and is planned to grow to 16 over the next five years. Its educational and research programs are enriched by integration with related activities in several departments in the Colleges of Engineering, Medicine, and Liberal Arts and Sciences. It participates actively in the campus-wide initiatives in Beckman Institute for Advanced Science and Technology, Institute for Genomic Biology, Coordinated Science Laboratory, Frederick Seitz Materials Research Laboratory, Micro and Nanotechnology Laboratory, etc.

The Head will hold the rank of Professor with tenure in the Department and will report directly to the Dean of the College of Engineering. Essential qualifications include a doctorate in a relevant field, recognition as a distinguished researcher and scholar, and knowledge of recent and future trends in bioengineering and related fields. Candidates are expected to present a clear plan to build a top notch Department through hiring of junior and senior faculty members.

Compensation for this position is competitive. To ensure full consideration, applications must be received by **April 14, 2006**. The proposed start date is August 16, 2006 or as soon as possible thereafter. Applicants should submit a letter of interest, a statement of vision for the development of the Department, a full resume including a list of publications, and the names, addresses, email addresses, and telephone numbers of five references to:**Professor K.C. Ting, Chair, Bioengineering Head Search Committee, C/O Katherine Darr, 306 Engineering Hall, MC-266, University of Illinois at Urbana-Champaign, 1308 West Green Street, Urbana, Illinois 61801; Phone: 217-333-3570; Fax: 217-244-0323; Email: kcting@uiuc.edu.**

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UNIVERSITY OF CALIFORNIA, BERKELEY
ASSISTANT PROFESSOR

The Department of Nutritional Sciences and Toxicology, the University of California-Berkeley, seeks an assistant professor for a nine-month tenure-track appointment starting as early as July 1, 2006. We expect the appointee to develop a vigorous and independent research program investigating diet, metabolism and chronic disease and/or toxicology related to dietary constituents. Note that this is a second position, distinct from our ongoing search in "metabolic regulation and/or the control of metabolic systems".

Applicants should have a bioscience Ph.D., M.D., or equivalent degree, with training and experience in experimental biology and/or toxicology. The appointee will have opportunity to work with Ph.D. students in four interdepartmental programs: Molecular and Biochemical Nutrition; Molecular Toxicology; Comparative Biochemistry; Endocrinology. The appointee also will contribute to educating undergraduates seeking degrees in nutritional biology and molecular toxicology.

Applications should include a curriculum vitae, a statement of current and proposed research, copies of publications, and the names and addresses of at least three references. Applicants should have their referees send references directly, and should refer their referees to the UC Berkeley Statement of Confidentiality at: **http://apo.chance.berkeley.edu/evalltr.html**.

Applications should be submitted to **DNSTsearch@Berkeley.edu** (electronic submissions strongly preferred) or to: **NST2 Search Committee Chair, Department of Nutritional Sciences and Toxicology, 119 Morgan Hall, University of California, Berkeley, CA 94720-3104**. Applications sent electronically or postmarked after **April 3, 2006** cannot be considered.

The University of California is an Equal Opportunity, Affirmative Action Employer. We are especially interested in a diverse applicant pool.

UNIVERSITY of CALIFORNIA
Riverside

FACULTY POSITION IN COSMOLOGY/PARTICLE ASTROPHYSICS
Department of Physics

The Department of Physics at the University of California, Riverside, is seeking an outstanding individual for a faculty appointment in the area of cosmology/particle astrophysics. This appointment will initiate a new program at UCR, which will complement existing programs in Astronomy, Astrophysics and Elementary Particle Physics. The appointment will be at the Assistant, Associate or Full Professor rank, as appropriate. The appointment will be effective July 1, 2006.

We encourage applications from candidates capable of instituting and sustaining a vigorous research program, and having an outstanding record of research achievement and leadership in one or more areas relevant to the field, such as dark matter or dark energy, structure formation, or the early universe. Candidates are also expected to support the training of graduate students and teach at the undergraduate and graduate levels. Salary will be competitive and commensurate with qualifications and level of appointment.

Applicants should submit curriculum vitae, list of publications, statement of research and teaching objectives, and names and addresses of four references. Applications should be directed to: **Chair, Cosmology Search Committee, Department of Physics, University of California, Riverside, 3401 Watkins Drive, Riverside, CA 92521-0413**

Review of applications will commence on **February 1, 2006**, but the position will remain open until filled. For more information please visit the UCR web site at **www.ucr.edu**, the College of Natural and Agricultural Sciences at **www.cnas.ucr.edu**, and the Department of Physics at **http://www.physics.ucr.edu/** .

The University of California is an Equal Opportunity Employer committed to excellence through diversity.

Faculty Position
Schepens Eye Research Institute, An Affiliate of Harvard Medical School

Schepens Eye Research Institute an affiliate of Harvard Medical School invites applications for a mid level faculty position. Successful candidates will be qualified for a Harvard Medical School appointment at the Associate Professor level. We are seeking candidates with a Ph.D. and/or M.D. or O.D. degree, with at least 5 years faculty status and an established ability to maintain independent research funding. Candidates with a strong publication record and demonstrated expertise in any of the following areas: biological imaging, cell and molecular biology, developmental biology, immunology, neuroscience, microbiology, low vision or those using an interdisciplinary approach to study vision or eye disease, are encouraged to apply. Successful candidates will be expected to have an independent research program with the objective of developing strategies that could prevent or treat blinding eye diseases. Candidates with expertise in the areas of age related macular disease, low vision, ocular surface/corneal disease, ocular infection, neural regeneration and diabetic retinopathy are particularly urged to apply. As Harvard Medical School Faculty Members, candidates will also be expected to teach trainees or students. Ample opportunities exist for fruitful interaction and collaboration with faculty members at The Schepens and other Harvard Medical School departments. More information about The Schepens, its mission, and faculty can be obtained at: **http://www.TheSchepens.org**.

Deadline for submission of applications is May 15, 2006. Applicants should submit their curriculum vitae, the names and addresses of three references, and a statement of the proposed research program to:

Ilene K. Gipson, Ph.D., Chair, Research Scientist Search Committee
Schepens Eye Research Institute
20 Staniford Street, Boston, MA 02114
Email: Gipson@vision.eri.harvard.edu

Equal Employment Opportunity/Affirmative Action Employer. M/F/V/H.

OHSU | OGI SCHOOL OF SCIENCE & ENGINEERING

Gordon and Betty Moore Endowed Chair in Biomedical Engineering

The OGI School of Science and Engineering at Oregon Health & Science University seeks candidates for an endowed Professorship. Applications are invited from distinguished scientists working in health-related areas of nanobiotechnology, as broadly defined. Applicants should have a record of significant academic accomplishment; preference will be given to those candidates with strong records of extramural funding, program development, and translational research.

The successful candidate will exhibit leadership skills, including the ability to recruit and mentor promising young faculty. He/she will be expected to build a program that will engage OHSU scientists and clinicians, state and regional organizations, and industrial partners. All areas of research will be considered; existing faculty strengths include neuroscience, cardiovascular disease, biomedical optics and imaging.

The Biomedical Engineering (BME) Department will soon move to a new building near the Willamette River in downtown Portland, Oregon. This location, connected to OHSU's main campus by an aerial tram, will anchor OHSU's emerging South Riverfront Campus — a focus for biotechnology development in Portland that will integrate OHSU and other regional academic, research and clinical activities.

Applicants should submit electronically a curriculum vitae, a statement of research and teaching interests and the names of six potential references to: **moore.chair.search@bme.ogi.edu**. Inquiries and requests for additional information may be sent to: **moore.inquiries@bme.ogi.edu**. All contacts will be held in confidence.

OHSU is an Affirmative Action, Equal Opportunity Institution

Molecular and Cellular Biologists

Molecular and Cellular Oncology

The Department of Molecular and Cellular Oncology at The University of Texas M. D. Anderson Cancer Center is seeking outstanding molecular and cellular oncologists. The department has openings for two full-time, tenure-track faculty with demonstrated excellence in molecular and cellular approaches to understanding the molecular mechanisms of cancer development. Although not required, the following expertise is encouraged: stem cell, biology, integrated biology or proteomic approaches to elucidate the molecular mechanisms that cause cancer. Incumbents will be responsible for establishing their own independent research and expected to write grants and papers. Applicants must have a doctoral degree, postdoctoral experience and be eligible to apply for federal grants.

Interested applicants should send a letter and curriculum vitae to:

Mien-Chie Hung, Ph.D.
Chair, Department of Molecular and Cellular Oncology, Box 108
The University of Texas M. D. Anderson Cancer Center,
1515 Holcombe Blvd., Houston, Texas 77030
E-mail: nedwards@mdanderson.org

THE UNIVERSITY OF TEXAS
MD ANDERSON
CANCER CENTER
Making Cancer History®

M. D. Anderson Cancer Center is an equal opportunity employer and does not discriminate on the basis of race, color, national origin, gender, sexual orientation, age, religion, disability or veteran status except where such distinction is required by law. All positions at The University of Texas M.D. Anderson Cancer Center are security sensitive and subject to examination of criminal history record information. Smoke-free and drug-free environment.

Dream. Challenge. Succeed.

BIOCHEMISTRY FACULTY POSITION

The Department of Molecular and Cellular Biochemistry invites applications for a tenure track faculty position at the Associate, or Full Professor level. Successful candidates must possess a Ph.D., M.D. or equivalent degree and an active, independent research program. We are seeking individuals to complement existing departmental programs including, but not limited to the areas of diabetes, cardiovascular disease, neuroscience, and cancer research, but we welcome all qualified applicants. Preference will be given to candidates with a proven track record of independent research and sustained extramural funding.

The successful candidate will benefit from a stimulating and collaborative environment within the department and a strong graduate program. Competitive start-up funds, salaries, state-of-the-art facilities and appropriate space will be offered in a new 185,000 ft² research building.

Evaluation of applicants will begin April 2006. Interested individuals should send their curriculum vitae, a brief statement of research plans and three references to:

MCB Faculty Search Committee
B278 Biomed. Biol. Sc. Res. Bldg.
741 South Limestone St.
Lexington, KY 40536-0509

UK
UNIVERSITY OF KENTUCKY

For further information about the Department, visit: **www.mc.uky.edu/biochemistry**

The University of Kentucky is an equal opportunity employer and encourages applications from minorities and women.

CHAVI
CENTER FOR HIV/AIDS VACCINE IMMUNOLOGY

The Duke Human Vaccine Institute, occupying a place of national and international leadership in the fight against major infectious diseases, currently has the following opportunities in its Center for HIV/AIDS Vaccine Immunology (CHAVI):

CHAVI CHIEF OPERATING OFFICER

The COO will assist the Director in all aspects of CHAVI and HVI operations. This position provides leadership, management, and vision, ensuring proper operational controls, procedures, and systems are implemented and followed. Key responsibilities include directing operational initiatives and collaborating with senior management on R&D strategic initiatives and product development goals. Candidates will have a minimum 10 years of management experience, reflecting a strong history of management experience in the medical affairs, clinical research and regulatory affairs arena. A doctoral level of education is preferred, though not mandatory.

CHAVI CHIEF MEDICAL OFFICER

The CMO is a faculty position responsible for building, organizing and directing the CHAVI in its entirety, including the HVI Clinical Research Department. The position has day-to-day oversight of all clinical research activities, as well as Medical Affairs, Biometrics, Regulatory/Safety and medical oversight of the HVI Business Development activities. Candidates will be MDs Board Certified in Infectious Diseases and preferably possessing 5-10 years of related academic and/or industry experience, especially in leading clinical research trials related to vaccine development. Clinical protocol writing experience and strong negotiation, presentation and networking skills are essential.

CHAVI CHIEF SCIENTIFIC OFFICER

The CSO provides the CHAVI with the leadership and vision necessary to spearhead the clinical discovery effort and ensure compliance with proper scientific methodology. A key part of the role is to manage CHAVI R&D activities while building partnerships and relationships with the scientific community. The CSO represents the CHAVI and HVI on scientific and technical matters related to pre-clinical R&D. Candidates must have doctoral level education with 5-10 years of related academic and/or industry experience. Outstanding scientific credentials are preferred. Background must include experience in scientific team management, bench research, research project management, and international partnerships.

To apply for a position, please submit your Curriculum Vitae and cover letter, via email, to: **w.smith@duke.edu** or mail to: **Duke Human Vaccine Institute, 114A Research Park I, Circuit Drive – MC 3258, Durham, NC 27710, Attention: William L. Smith – HR Manager**. Detailed job descriptions available upon request to William Smith at w.smith@duke.edu. Duke University Medical Center is located in the energetic and progressive Research Triangle area of North Carolina.

Duke University is an Equal Opportunity/ Affirmative Action Employer.

Duke University
Medical Center

SALK INSTITUTE
FOR BIOLOGICAL STUDIES

Crick-Jacobs Center for Theoretical and Computational Biology

The Crick-Jacobs Center for Theoretical and Computational Biology, named in honor of Salk Nobel Laureate, Francis Crick, is an interdisciplinary research unit at the Salk Institute. The overall goal of the Center is to integrate experimental and theoretical approaches to understanding the organization of signaling systems and the functional neuroanatomy of the brain, from the molecular to the systems levels, and how behavior arises from the interactions between the brain's many components. The scientists who work at the Crick-Jacobs Center combine approaches from biology, physics, chemistry, mathematics, computer science, and engineering and exploit techniques that include computer simulations, imaging, viral vectors, and molecular genetics.

Junior Fellows

Junior Fellows of the Crick-Jacobs Center work on projects that involve two or more laboratories at the Salk Institute and include both experimental and theoretical approaches. The appointment is for three years and includes internal funding for carrying out the research.

Some of the specific problems that Junior Fellows might study include:

(1) Systems and networks regulated by transcriptional complexes in neuronal differentiation and stem cell maintenance;
(2) Developing methods for reversibly silencing specific neural types in vivo and applying this to understanding perception and cognition in monkeys;
(3) Optical recording of activity in C. elegans during behavior.

Applicants for Junior Fellows should identify faculty at the Salk in a cover letter, with whom they intend to work and submit a 3 page summary of the proposed project, curriculum vitae, and 3 letters of recommendation to the address below.

Faculty

The Salk Institute invites applications for faculty positions at any level in the Crick-Jacobs Center for Theoretical and Computational Biology. Appointees will be expected to establish independent research programs aimed at understanding the organization of signaling systems and the functional neuroanatomy of the brain, from the molecular to the systems levels, and how behavior arises from the interactions between the brain's many components. Present members of Crick-Jacobs Center, and their research interests, are **Terrence Sejnowski** (computational neuroscience), **Sydney Brenner** (functional networks of genes and cells), **Charles Stevens** (synaptic physiology and theoretical neuroanatomy), and **Ed Callaway** (thalamic and cortical networks). Qualified candidates are invited to submit curriculum vitae, description of present and future scientific endeavors, and 3 letters of recommendation.

The Salk Institute offers a highly interactive environment with a number of research groups working in areas of genetics, molecular biology and neuroscience. The Salk Institute offers a competitive salary and excellent benefits. Applications will be reviewed as they are received, and will be accepted until the positions are filled. Forward applications to: **Chair, Crick-Jacobs Search, The Salk Institute, 10010 North Torrey Pines Road, La Jolla, CA 92037, USA.** Applications may also be submitted electronically to: **murray@salk.edu**.

www.salk.edu **http://crick-jacobs.salk.edu**

ASSOCIATE DEAN FOR CLINICAL AND TRANSLATIONAL SCIENCES

UMDNJ-Robert Wood Johnson Medical School seeks applications for the position of Associate Dean for Clinical and Translational Sciences. The successful candidate must have an MD and/or Ph.D. degree, and academic credentials that would qualify for appointment at the Associate Professor or Professor level. A successful track record in academic clinical/translational research and a history of peer-reviewed research funding are essential. Experience in clinical research administration is desirable. The candidate must possess strong interpersonal and leadership skills, and it is anticipated that the individual will remain active in clinical/translational care/research. A strong vision to promote and enhance excellence and diversity in all aspects of clinical and translational sciences is crucial for success in this position.

Please send nominations and/or applications, including a brief statement of the attributes and qualities of the applicant, and curriculum vitae to: **Dr. Kathleen Scotto, Sr. Associate Dean for Research, UMDNJ-Robert Wood Johnson Medical School, CINJ Room 4564, 195 Little Albany Street, New Brunswick, NJ 08903-2681 or e-mail scottoka@umdnj.edu.** UMDNJ is an AA/EO Employer, M/F/D/V. For more information, visit **www.umdnj.edu/hrweb.**

UMDNJ
ROBERT WOOD JOHNSON MEDICAL SCHOOL
University of Medicine & Dentistry of New Jersey

University of Missouri Health Care

Research Faculty Position

The University of Missouri School of Medicine Department of Surgery is seeking outstanding candidates for two research faculty positions. Rank and appointment status are contingent upon qualifications.

The candidate should have experience in a field of research in which surgeons interface. This includes, but is not limited to, cancer, heart disease, inflammation, wound healing, trauma, and vascular biology. Candidate must demonstrate evidence of current or future potential for federal funding, show interests in teaching medical students and surgical residents, and have the desire to interact with surgical researchers in developing a vigorous, extramurally funded research program.

Applicants should send curriculum vitae to: **Steve Eubanks, M.D., Chairman, Department of Surgery, University of Missouri-Columbia, Health Sciences Center, One Hospital Drive, Columbia, Missouri 65212.**

Equal Opportunity/ Affirmative Action/ ADA Employer

Visit the Department of Surgery's Web site at http://www.surgery.missouri.edu/. Go to "Practice Opportunities".

ATSU
A.T. STILL UNIVERSITY

ASSISTANT OR ASSOCIATE PROFESSOR, PHARMACOLOGY

Tenure-track, full-time position that is 100% fully funded by the Institution. Applicants must have a Ph.D. in pharmacology or a related biomedical discipline, or a D.O. or an M.D. degree, and two years of postgraduate academic experience with evidence of productivity. Training in pharmacology is preferred, but other fields will be considered. The candidate will be expected to participate in the medical pharmacology educational program, which involves teaching and mentoring medical students in an integrative and developing Team-Based Learning curriculum, and biomedical science graduate students in the Biomedical Sciences Graduate Program. The candidate will also be expected to establish a productive program of independent scholarly activity in an appropriate arena which would be eligible for support by external funding. The candidate would also be expected to serve on academic committees, be involved in mentoring activities, and participate in the academic community as appropriate for a faculty member. Candidates wishing to be considered for this position should send a curriculum vitae, list of publications and scholarly work, summary of teaching experience, statement of professional goals, and names of three professional references to: **Dr. Robert J. Theobald, Chairperson, Department of Pharmacology, Kirksville College of Osteopathic Medicine, A.T. Still University of Health Sciences, 800 West Jefferson Street, Kirksville, Missouri 63501**. Applications will be accepted until the position is filled. The target date for filling the position is August 1, 2006. *A.T. Still University of Health Sciences is an EEO Employer.*

Plastic Surgery Research Scientist (Doctoral Level)

We are interested in further development of a multidisciplinary laboratory effort in plastic surgery. The laboratory is focused on normal and abnormal physiologic processes in craniofacial biology, nerve physiology, and wound healing. Currently we have two ongoing NIH grants in the area of craniofacial biology and nerve physiology. We are currently seeking a qualified scientist to assume a central role in these activities. This scientist will have joint sponsorship by the Departments of Plastic Surgery, Physiology, and Cell Biology, Neurobiology and Anatomy. Qualifications would include a Ph.D. in molecular or developmental biology with specific experience and skills in the area of transgenic research. This position is a full-time academic faculty post, with rank commensurate with experience. The overarching goal is to advance the understanding of craniofacial biology at a molecular and cellular level to elucidate specific pathologic processes and translate these findings into improved or even novel therapeutic intervention. Applicants should anticipate involvement in collaborative and NIH supported programmatic research that would include both clinical and basic scientists with common interests in these problems. Competitive salary support is provided for the first two years, but contribution by the candidate is expected in the form of independent, peer-reviewed grant support over time.

Interested candidates should submit their C.V. and three letters of reference to: **Hani S. Matloub, M.D., Department of Plastic Surgery, The Medical College of Wisconsin, 8700 Watertown Plank Road, Milwaukee, WI 53226**.

Assistant/Associate Professor
Pharmacokinetics
School of Pharmacy

The University of Southern California Department of Pharmaceutical Sciences (**http://www.usc.edu/schools/pharmacy/graduate/pharmsci/**) invites applications for an Assistant/Associate Professor position, tenure-track or tenured. The successful candidate should have a doctoral degree and, preferably, postdoctoral experience, and is expected to develop a strong research program with extramural funding that complements and expands existing departmental strengths in epithelial cell biology, membrane trafficking, protein drug delivery, molecular modeling and genetic engineering in drug design, and pharmacokinetic imaging. Candidates with research interests in pharmacokinetics and pharmacodynamics of agents ranging from small molecules to macromolecular drugs are particularly encouraged to apply.

The University of Southern California offers cutting-edge opportunities for multidisciplinary and translational research collaborations, including an NCI-designated Comprehensive Cancer Center, an NIH-sponsored Liver Center, the NIH-sponsored Doheny Eye Institute, the USC Initiatives in Biomedical Imaging Science and in Nanotechnology, a Program Project in Biomedical Engineering including PK/PD modeling and access to one of the widest variety of affiliated private and public hospitals (**http://www.usc.edu/health/ClinHospPharm.html**) in the United States.

Candidates should send the names of three references, curriculum vitae, and a summary of research accomplishments and future goals to: **Sarah Hamm-Alvarez, Ph. D., Chair, Pharmaceutical Sciences Search Committee, University of Southern California School of Pharmacy, 1985 Zonal Avenue, Los Angeles CA 90033** or email: **shalvar@usc.edu**. Review of applications will begin immediately, and will continue until the position is filled.

The University of Southern California is an Equal Opportunity/ Affirmative Action Employer and encourages applications from women and minorities, and provides reasonable accommodation to individuals with known disabilities.

Director

Institute of Biological Chemistry
Academia Sinica, Taipei

Academia Sinica, Taiwan, invites applications and nominations for the position of Director of Institute of Biological Chemistry (IBC). The initial appointment is for a period of three years (renewable for a second term), and will also carry the title of Research Fellow.

Academia Sinica is the pre-eminent academic institution in Taiwan. It is devoted to basic and applied research in mathematics and physical sciences, life sciences, and humanities and social sciences. IBC engages in interdisciplinary research bridging chemistry and biology, with particular emphasis on structural biology, proteomics, glycobiology, differentiation and development and signal transduction. The institute is well funded and equipped with modern research facilities. For details about Academia Sinica and IBC, please consult the website: **http://www.sinica.edu.tw**

Interested candidates should have a Ph.D. degree, a distinguished record of academic scholarship, and diverse experience in university and professional service. He/she is expected to pursue a vigorous research program. The successful candidate will be expected to build on the existing strengths of the Institute, develop new research thrusts, promote basic life sciences and provide intellectual leadership in basic and applied life sciences in Taiwan.

Applications and nominations, including a full curriculum vitae, a publication list, and three letters of recommendation, should be submitted to **Vice President, Academia Sinica, 128 Academia Road Section 2, Nankang, Taipei, 115, Taiwan.** Screening of applications/nominations will begin immediately, and will continue until the position is filled.

SIMON FRASER UNIVERSITY

Canada Research Chairs
Aquatic Conservation in the Department of Biological Sciences

The Department of Biological Sciences seeks to appoint two tenure-track Tier II Canada Research Chairs in Aquatic Conservation. Candidates should have a strong international track record in research on conservation of marine or freshwater ecosystems. Areas of research interest could include fisheries, anthropogenic impacts on habitats, ecosystem dynamics, land-water linkages, and the ecology of threatened species. Appointment will be at the Assistant or Associate Professor level. Successful candidates will be nominated by the university through the Canada Research Chair program **http://www.chairs.gc.ca/**. The positions will benefit from a strong research environment with more than 15 faculty members with common interests in aquatic ecology, conservation and management including the newly established Tom Buell BC Leadership Chair in Salmon Conservation **http://www.sfu.ca/biology/faculty/reynolds/**.

Review of applications will begin on **March 31 2006**, and the search will remain active until the positions are filled. Applicants should send a curriculum vitae, three representative reprints, a two-page summary of their research objectives and teaching philosophy, and three letters of reference to:

Dr. Tony D. Williams, Chair
Department of Biological Sciences
Simon Fraser University
8888 University Blvd.
Burnaby, B.C. V5A 1S6
Canada
E-mail: tdwillia@sfu.ca
FAX: 604 291-4312

All qualified candidates are encouraged to apply; however, Canadians and permanent residents will be given priority. The appointment is subject to final budgetary approval by the University.

Simon Fraser University, located in the greater Vancouver area, is committed to employment equity, welcomes diversity in the workplace, and encourages applications from all qualified individuals including women, members of visible minorities, aboriginal persons, and persons with disabilities.

DIRECTOR, DIABETES CENTER

The University of Alabama at Birmingham is seeking applications and nominations for the position of Director of a new UAB Diabetes Center. The successful candidate should be nationally recognized as a leader with interest in Type I diabetes research and demonstrated excellence in both research and administration. The Director will be expected to provide inspired leadership and develop strategic concepts for the Center in conjunction with a strategic plan for the School of Medicine that envisions a world-class diabetes translational and basic research initiative. The appointment will be as Professor or Associate Professor with tenure in an academic department most suited to the candidate's background, training and research interests.

Resources include a series of major philanthropic gifts, an endowed chair, and approx. 20,000 gross square feet of laboratory space dedicated to diabetes research in the new Shelby Research building. The recruitment is being conducted in parallel with growth of a diabetes clinical care program and a new diabetes clinic. Outstanding research programs in related areas at UAB include basic studies of obesity, islet cell biology, clinical physiology, genetics, immunology, vascular biology, and lipid science. The institutional capabilities also include access to human pancreatic tissue, islet cell isolation, GMP grade islet cells, and a successful islet transplantation program. A large diabetes patient base (both Type 1 and Type 2 diabetes) represents a powerful platform from which to initiate a clinical trials unit.

UAB is a comprehensive urban University and Medical Center enrolling 16,000+ students in 12 schools on its 80 block campus. It has extramural research awards of over $260 million and is categorized by the Carnegie Foundation as a Doctoral/Research-Extensive University. The School of Medicine is ranked 18th in NIH funding (2004). The University is the state's largest employer with more than 18,000 employees and a $1.2 billion budget. Nominations and applications should include a curriculum vitae, bibliography, and the names and addresses of at least three references and should be submitted electronically (preferably) or mailed to:

Eric J. Sorscher, M.D.
Chair, Diabetes Center Director Search Committee
Professor of Medicine, Hematology and Oncology
c/o Josephine Jackson-Banks
1530 3rd Avenue South
1203 Faculty Office Tower
Birmingham, AL 35294-3412
jjbanks@uab.edu

The University of Alabama at Birmingham is an Affirmative Action/Equal Opportunity Employer.

Immunology Tenure Track Faculty Position

The Department of Immunology at the University of Connecticut Health Center seeks outstanding investigators for a tenure-track position at the Assistant/Associate Professor level. Although all areas of immunology will be considered, we are particularly interested in individuals using molecular and cellular approaches to study immune system function in vivo. Areas of priority include dendritic cell biology or innate immunity, immune cell signaling and immunity to infection. Salary and start-up funds are highly competitive and outstanding core facilities are available. Applicants must have a Ph.D., D.Sc. and/or M.D. with postdoctoral experience and a quality publication record. For the Associate Professor level, applicants should have a record of substantial productivity and sustained extramural funding.

Please submit curriculum vitae, two-page summary of research interests and the names of three references to: **Leo Lefrançois, Ph.D., Chair, Immunology search committee, Dept. of Immunology MC1319, UCONN Health Center, 263 Farmington Ave., Farmington, CT 06030-1319**. Email: **llefranc@neuron. uchc.edu**. For further information on UCHC, please visit **immune.uchc.edu**.

UCHC is an Equal Opportunity Employer M/F/V/PwD.

www.admin.cam.ac.uk/jobs/

On 1 January 2006 the Departments of Anatomy and Physiology merged to form a new Department of Physiology, Development and Neuroscience consolidating research and teaching strengths, and creating dynamic synergies within and across themed research groups.

Substantial refurbishment of the Department's research and teaching spaces is well advanced and the University has agreed to fund several new posts. A key aspiration of the new Department is to build world-class research groups at the cutting edge of the science that links genes, cells and tissues in the study of physiology, development and neuroscience.

As part of this exciting initiative, two Professorships are available for appointment as soon as possible for established outstanding scientists to lead research teams in the fields of Integrative Biology and Cellular Physiology.

The Herchel Smith Professorship of Molecular Biology

The Board of Electors to the Herchel Smith Professorship of Molecular Biology invite applications for this Professorship from persons whose work falls within the field of Integrative Biology.

The Professorship of Physiology

The Board of Electors to the Professorship of Physiology invite applications for this Professorship from persons, whose work falls within the field of Cellular Physiology.

Informal enquiries about both posts may be made to Professor Bill Harris, tel: (01223) 333814. E-mail: harris@mole.bio.cam.ac.uk

Further information on both posts may be obtained from the Academic Secretary, University Offices, The Old Schools, Cambridge CB2 1TT, (e-mail: ibise@admin.cam.ac.uk), to whom a letter of application should be sent, together with details of current and future research plans, a curriculum vitae, a publications list and form PD18 with details of two referees. Closing date: 31 March 2006.

UNIVERSITY OF CAMBRIDGE

The University offers a range of benefits including attractive pension schemes, professional development, family friendly policies, health and welfare provision, and childcare. The University is committed to equality of opportunity.

KU CANCER CENTER
The University of Kansas

PROGRAM LEADER FOR CANCER BIOLOGY

The University of Kansas Cancer Center invites applications for a senior-level tenure-track faculty position to help lead the basic science programs of the cancer center. Applicants should have an MD, MD/PhD, Ph.D. or equivalent degree. The successful applicant is expected to have an externally funded research program compatible with existing cancer center strengths in cellular or molecular biology, genetics, signal transduction, drug development or other projects related to the causes, progression, prevention, diagnosis, or treatment of cancer. (Refer to **http://www.kumc.edu** or **http://kmcri.kumc.edu**) Current cancer center programs include: Cancer Biology, Cancer Risk Assessment, Prevention and Control, Experimental Therapeutics, and Clinical Research. Participation in the educational missions of the medical and graduate schools is also expected.

The University of Kansas is making a major commitment to growing the cancer program and has targeted addition of faculty and enhancement of facilities as the cancer center moves toward NCI designation. Competitive salary and start-up package along with excellent research facilities and opportunities are available.

Send CV, cover letter describing interest in position and three references to: Electronic Files (preferred): **Susan Harp** at **sharp@kumc.edu**, or Hard Copy to: **Chair, Search Committee, Program Leader of Cancer Biology, University of Kansas School of Medicine, MS 1027, 3901 Rainbow Blvd, Kansas City, KS 66160-7300**.

AA/EOE

CHAIR
DEPARTMENT OF ANATOMY AND CELL BIOLOGY

THE UNIVERSITY OF NORTH DAKOTA

The University of North Dakota School of Medicine & Health Sciences invites applications and nominations for the position of Chair of the Department of Anatomy & Cell Biology. We seek an outstanding medical scientist with a strong research record, including extramural support, and a commitment to excellence in teaching undergraduate, graduate and professional medical and allied health students. The candidate will be expected to complement, expand and strengthen existing areas of research in the department and may utilize the state-of-the-art imaging facilities, including a Zeiss LSM 510 META confocal system and a Hitachi 7500 TEM and 4700 field emission SEM. A new MicroPET and Cyclotron, a dedicated small animal research facility and a Genomics and Proteomics facility are available. The applicant should possess strong interpersonal and leadership skills in mentoring faculty, directing students and performing administrative duties.

The Chair will oversee a department whose active research interests include neurosciences, cell and cancer biology. The department offers M.S., Ph.D., and M.D./Ph.D. degrees and numerous traditional anatomical courses at the graduate level, instructs first and second year medical students, and provides undergraduate courses to allied health and non-majors. Further information is available at: **http://www.med.und.nodak.edu/depts/anatomy/**

The University is located in Grand Forks and currently enrolls over 12,000 students. Grand Forks is a family-friendly community in a region offering excellent and rapidly expanding cultural, recreational, and sporting activities. To learn more about the University of North Dakota and Grand Forks visit:
http://www.und.edu and http://www.grandforksgov.com.

Review of applications will begin April 1, 2006 and the search will remain open until the position is filled. Applicants should submit a detailed curriculum vitae, a letter of interest outlining prior experience, research interests, teaching philosophy, plans for the future and the names and addresses of three references to:

Dr. Joshua Wynne, Professor of Internal Medicine, Executive Associate Dean, Associate Dean for Academic Affairs and Chair of the Search Committee, University of North Dakota School of Medicine & Health Sciences, Box 9037 Grand Forks, North Dakota 58202-9037

(The University of North Dakota is an Equal Opportunity/Affirmative Action institution.)

COLUMBIA UNIVERSITY
IN THE CITY OF NEW YORK

Theoretical Neuroscience Faculty Recruitment

The Center for Theoretical Neuroscience at Columbia University is recruiting for a faculty position in theoretical and computational neuroscience. Candidates who apply mathematical analysis and computer simulation to topics in neuroscience at levels ranging from cellular to systems and cognitive are urged to apply. We encourage applications for a tenure-track Assistant Professor position, but will also consider applications from more senior investigators for tenured positions.

The Center for Theoretical Neuroscience is a highly interactive group of faculty (four full-time and one part-time, at this point), postdoctoral researchers, and graduate students who also interact extensively with experimentalists within Columbia's well-known program in neurobiology and behavior as well as with members of the scientific departments at the Morningside Heights campus. These interactions will be augmented in the upcoming years by the new Columbia Neuroscience Initiative, which is hiring a significant number of new faculty in the area of circuit-level neuroscience.

Applications for this position are requested by March 31, 2006. A CV, cover letter including statement of interests, and three letters of reference under separate cover should be e-mailed care of **Andrew Fink, andrew@neurotheory.columbia.edu.** In addition, please mail a hard copy of these documents to:

Theoretical Neuroscience Search
c/o Andrew Fink
Columbia University
College of Physicians and Surgeons
Kolb Research Annex
1051 Riverside Drive
New York, NY 10032-2695

Columbia University takes affirmative action to ensure equal employment opportunity.

Mathematical Biology Faculty
LOYOLA MARYMOUNT UNIVERSITY

The College of Science and Engineering seeks candidates for a Presidential Professorship in Mathematical Biology. Candidates must have a distinguished record in teaching and research and a clear vision for providing leadership in interdisciplinary educational and research programs in Mathematical Biology. The ideal candidate will receive a joint appointment in Biology and Mathematics at the rank of Professor. Our College's faculty have a variety of current and emerging research interests, including bioinformatics, coding theory, dynamics, ecology and evolution, epidemiology, genomics, knot theory, modeling, proteomics, and probability and statistics. The individual we are seeking will broaden and complement our current interests and expertise. LMU currently maintains an individualized studies undergraduate degree in Biomathematics, and we are actively developing a formal major. Our College currently participates in two REU programs, with more under development. The successful candidate will provide leadership not only in the undergraduate degree programs and any future initiatives but also the recruitment of additional faculty to strengthen interdisciplinary interactions among departments in the College.

Requirements for the position include a Ph.D. in Biology, Mathematics, or a relevant, related discipline. Applicants are requested to send a letter of application, curriculum vitae, vision statement for the position, and three letters of reference. Review of applicants will commence on **May 1, 2006**, and continue until the position is filled. Materials should be sent to: **Biomathematics Search Committee, Department of Mathematics, UH 2700, Loyola Marymount University, 1 LMU Drive, Los Angeles, CA 90045-2659. For additional information, contact Dr. Ben Fitzpatrick, (310) 338-7892**. To learn more about LMU and the College, visit **www.lmu.edu** and **cse.lmu.edu**.

Loyola Marymount, a comprehensive university in the mainstream of American Catholic higher education, seeks professionally outstanding applicants who value its mission and share its commitment to academic excellence, the education of the whole person, and the building of a just society. LMU is an Equal Opportunity Institution actively working to promote an intercultural learning community. Women and minorities are encouraged to apply.

City of Hope®
Where the Power of Knowledge Saves Lives®
www.cityofhope.org

The Beckman Research Institute of City of Hope, one of the nation's most prestigious research and cancer treatment centers, believes in the power of world-class research and its impact on people all over the globe

Post Doctoral Fellows:

- **Marcia Miller, Ph.D.; mamiller@coh.org; Molecular Biology**
  An NSF funded project focused on proteomic profiling and function of NK cells in Gallus gallus. **Job Code# 41948**.
- **Don J. Diamond, Ph.D.; jsantos@coh.org; Virology**
  Research in viral immunology in support of a project on CMV vaccines using a mouse challenge model of recombinant MCMV expressing HCMV antigens. **Job Code# 41850**.
- **Steve S. Sommer, M.D., Ph.D.; sommeradmin@coh.org; Molecular Genetics**
  Testing the lipophilic mutation hypothesis of breast cancer with a novel method able to measure mutation load in human and mouse breast tissue. **Job Code# 40419**.
- **Innovative mutation detection and analysis methods** to support a Genetic Epidemiology and Molecular Diagnostic Lab. **Job Code# 42085**.
- **Distinguished Fellow in Molecular Epidemiology of Cancer/Complex Disease:** Training opportunity for an outstanding applicant with City of Hope, clinical mentor and a laboratory mentor chosen by applicant. Applicants should have M.D., or M.D., Ph.D. Attractive stipend. **Job Code# 41029**.

To apply, applicants should contact the principal investigator at their designated e-mails above. For further information on any of our positions please visit our employment website **http://www.cityofhope.org/Employment/**, "click" Research Fellows/Post Docs and use the job code to locate your position of interest.

We offer competitive salaries, benefits, compensation and a great environment in which to work. To learn more, visit our website at **http://bricoh.coh.org/**.

Beckman Research Institute of the City of Hope, 1500 E. Duarte Road, Duarte, CA 91010-3000.

EOE/AA

FACULTY POSITIONS

National Yang-Ming University Bioscience Faculty Positions and Professorships teaching Basic Science

National Yang-Ming University **(http://www.ym.edu.tw/)** invites applications for research-oriented professorships and experienced professors to teach undergraduate basic science. Sabbatical visitors are welcome to apply.

I. Research-oriented faculty members will be recruited in the following areas of interest:
- Neuroscience
- Genomics and proteomics
- Biophotonics
- Medical informatics
- Cancer biology
- Bioscience areas that can be linked to the above research topics

The appointments are flexible. A faculty dormitory, or equivalent accommodation, and a round-trip airplane ticket will be provided. The salary and benefits will be equivalent to our regular appointments except for an optional retirement allowance. Salary supplementation will be subject to negotiation.

II. The teaching faculty positions are for biology, chemistry and physics in English. The appointments will be on an annual contract that is subject to review. Senior professors or those who are planning to retire in their home country are welcome to apply.

Interested applicants should submit a full curriculum vita and two recommendation letters.
Applications should be sent to:
Prof. Yau-Huei Wei, National Yang-Ming University, Taipei,Taiwan, Republic of China
Tel: 886-2-2826-7003 Fax: 886-2-2827-0199
E-mail: joeman@ym.edu.tw

Laboratory Manager
South Florida Water Management District
West Palm Beach, Florida

The South Florida Water Management District (SFWMD) is a regional agency of the state of Florida, and is charged with managing and protecting water resources of the region by balancing and improving water quality, flood control, natural systems and water supply. SFWMD's boundaries extend from central Florida to Lake Okeechobee, and from coast to coast, from Fort Myers to Fort Pierce, south through the sprawling Everglades to the Florida Keys and Florida Bay.

We have an exciting opportunity for a Laboratory Manager to provide leadership and direction to the Chemistry Laboratory Unit of the Water Quality Analysis Division. This position will lead unit operations to carry out a number of mandated and permit required activities and emergency water quality data analysis related to emergency events, litigation matters and high level policy issues. The position has full technical responsibility to interpret, organize, execute and coordinate assignments. Lead and manage laboratory staff (professional chemists and laboratory analysts), while influencing performance by creating an environment in which people can realize and express their capabilities and, in so doing, contribute more fully. Monitor, analyze, and evaluate work flow and productivity of the laboratory; maintain high data quality and ensure final release of laboratory data; procure all laboratory chemicals, supplies, instruments, or equipment; and plan and manage the laboratory budget. Represent the District as the prime contact on projects involving the laboratory. Interacts with senior District staff on significant technical matters. Stays current with technology. Work is performed in a laboratory where employees routinely use numerous chemical compounds, reagents, gases, acids, and bases. Typically has Masters Degree in chemistry or natural sciences and 8 - 10 years experience with extensive technical expertise in developing, implementing and evaluating new or improved methods for metals, nutrients and physical parameters including four (4) years experience leading and managing professional and technical staff. Job reference number 206142.

Please visit our website for more information and APPLY ONLINE at www.sfwmd.gov.

SOUTH FLORIDA WATER MANAGEMENT DISTRICT
Attn: Human Resources, P.O. Box 24680
West Palm Beach, FL 33416-4680
EOE

sfwmd.gov

CHEMNITZ UNIVERSITY OF TECHNOLOGY

International Research Training Group "Materials and Concepts for Advanced Interconnects"

Starting on **1 April 2006**, an International Research Training Group on "Materials and Concepts for Advanced Interconnects", jointly sponsored by the German Research Foundation (DFG) and the Chinese Ministry of Education, will be established between Chemnitz University of Technology, Fraunhofer Institute IZM Chemnitz, Berlin Technical University, Fudan University (Shanghai) and Shanghai Jiao Tong University.

At Chemnitz University of Technology, Berlin Technical University and Fraunhofer Institute IZM Chemnitz,

14 PhD positions and 1 Postdoctoral position

are open.

The International Research Training Group (IRTG) is concerned with new materials and concepts for metallization systems for ultra-large scale integrated circuits. Topics of the IRTG are chemical and physical aspects of materials along with their application and integration in microfabrication. We therefore welcome applications of highly qualified graduates in the following disciplines: Electrical Engineering / Microelectronic Engineering, Physics, Chemistry, Materials Science, and adjacent fields. A strong interest for multi-disciplinary research is required.

Please refer to the web page

http://www.zfm.tu-chemnitz.de/irtg/

for details. The application deadline is **31 March 2006**.

Korea Institute of Science and Technology
Research Opportunities (Senior Researcher & Researcher)

KIST is a top-notch research institute contributed by the government, and the only multi-disciplinary institute in South Korea. With its vision of becoming a globally outstanding institute, KIST has developed five major strategic research areas, each of which is designed to fulfill important scientific and industrial needs, as well as provide important benefits for society at large. KIST invites highly qualified researchers from all over the world in the following:

1. Research Fields(Detailed Fields): (A)KIST: (1)Spintronics, Microsystem(Nanophotonics or Nanodevice Fabrication, MEMS, Others) **(2)Functional Materials, Nanomaterials, Control of Materials Properties**(Organic Optoelectronic Materials, Functional Ceramic Materials & Thin Film, Organic/Inorganic Nanomaterials & Process, Solid State Physics, Surface Modification, Ion Beam Accelerator Engineering, Others) **(3)Human Computer Interaction, Robot**(Intelligent Software/User Modeling, Multi-Modal Interface/Context Awareness, Humanoid, Robot Intelligence, Others) **(4)Sustainable New Energy, Toxic Substances** (Fuel Cells & Secondary Batteries, Hydrogen Energy Production & Separation, Toxic Substance Control & Treatment, Others) **(5)Biologically Active Substances**(Molecular Combinatorial Chemistry, Neuro Biology, Molecular Imaging, Others). **(B)KIST-Gangneung Institute: Natural Products**(Natural Products, Analytical, Organic Chemistry, Pharmacognosy, Pharmacology, Life Sciences).

2. Application Requirement: (1)M.S. or Ph.D. corresponding to the above Research Fields (Detailed Fields), (2)KIST-Gangneung Institute: Applicant must be able to work in Gangneung city.

3. Selection Process: (1)Phase one: Review on Application **(2)Phase two**: Presentation on applicant's research theme and Interview **(3)Phase three:** Personal History Verification & Medical Check.

4. Required Documents: (1)Phase one: Application(1 original copy, use prescribed KIST Form, downloadable from http://www.kist.re.kr/En/index.asp), Transcript classified by Degree(1 original copy each) **(2)Phase two**: Resume(1 copy), Essay(Title: Field of Interest & Future Research Plans, 1 copy), Results of accomplished research(1 copy each), Signed Recommendation of Advisor(1 original copy, Ph.D. Only), Micro Soft Power Point for Presentation(Title: Research Field & Future Research Plans, Submit it by email.(recruit@kist.re.kr)) **(3)Phase three:** Personal History Report (3 original copies), Photo(6 original copies), Other Documents for Personal History Verification, Result of Medical Check.

5. Terms of Submission for Phase one : Mar. 01, 2006 ~ Mar. 31, 2006 (Submit by air mail, Only Applications received on or before the closing date will be accepted)

6. Place of Submission : Human Resources Management Team, KIST, 39-1 Hawolgok-dong, Seongbuk-gu, Seoul, Korea (Zip Code : 136-791)

7. For More Information : Home Page(http://www.kist.re.kr/En/index.asp), Tel(+82-2-958-6088), Email(recruit@kist.re.kr)

THE WESTAIM-ASRA CHAIR IN BACTERIAL BIOFILM RESEARCH

UNIVERSITY OF CALGARY

The **University of Calgary Faculty of Medicine** invites applications and nominations for a full-time academic position as the Westaim-ASRA Chair in Bacterial Biofilm Research.

The successful candidate will be a senior established investigator in biofilm research related to human or veterinary bacterial pathogens, with academic qualifications commensurate with an appointment at the rank of professor. The incumbent will also foster collaborative links with the Westaim Chair in Biofilm Research in the Faculty of Engineering and other biofilm researchers at the University of Calgary, and support a collaborative multidisciplinary research environment, as well as help attract outstanding students, research associates, and faculty to the University of Calgary.

With over 400 full-time members, the Faculty of Medicine is a leader in health research with an international reputation for excellence and innovation. A new research facility slated to open in 2006 will allow researchers to investigate scientific questions collaboratively in a unique setting that facilitates multidisciplinary studies with state-of-the-art investigative tools. Calgary is a vibrant, multicultural city of ~1,000,000 near the Rocky Mountains, Banff National Park and Lake Louise.

Please submit a curriculum vitae, a statement of research interests and goals, and the names of three referees by **April 15, 2006,** to:

Dr. Pamela A. Sokol, Chair
Selection Committee
Faculty of Medicine
University of Calgary
3330 Hospital Drive N.W.
Calgary, AB T2N 4N1

In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada. The University of Calgary is committed to employment equity.

PG Proudly Pr

www.ucalgary.ca

Department of Health and Human Services
National Institutes of Health
Office of the Director

Director, Office of Portfolio Analysis and Strategic Initiatives

The Office of the Director, National Institutes of Health (NIH) in Bethesda, Maryland, is seeking a Director for the new Office of Portfolio Analysis and Strategic Initiatives (OPASI). If you are an exceptional candidate with an M.D. and/or Ph.D. and the vision and ability to integrate science across multiple disciplines, we encourage your application.

As the OPASI Director, you will be responsible for the executive leadership for the coordination of overall NIH research portfolio analysis and strategic initiatives that fall within the OPASI's scope. The OPASI's primary objective is to develop: a transparent process of planning and priority-setting characterized by a defined scope of review with broad input from the scientific community and the public; valid and reliable information resources and tools, including uniform disease coding and accurate, current and comprehensive information on burden of disease; an institutionalized process of regularly scheduled evaluations based on current best practices; the ability to weigh scientific opportunity against public health urgency; a method of assessing outcomes to enhance accountability; and a system for identifying areas of scientific and health improvement opportunities and supporting regular trans-NIH scientific planning and initiatives. You will serve as the principal advisor to the NIH Director on issues involving OPASI's planning, analysis, and policy formulation and implementation activities, including efforts to strengthen trans-NIH strategic planning and funding, and improve data quality and develop analytical techniques for assessing the NIH research portfolio.

This position requires that the OPASI Director exercise leadership, initiative, and creativity in establishing and maintaining relationships with key Federal and non-Federal officials, nationally and internationally recognized scientific leaders and officials of academic, research, and other institutes and organizations, and professional and advocacy groups.

Salary is commensurate with experience; a full package of benefits (including retirement, health, life, long term care insurance, Thrift Savings Plan participation, etc.) is available.

Applications for this position will be reviewed by a Search Committee chaired by Dr. Jeremy Berg, National Institute of General Medical Sciences and Dr. Elizabeth Nabel, Director, National Heart, Lung and Blood Institute.

Interested applicants should send a Curriculum Vitae to **Ms. Teresa Leary, 31 Center Drive – MSC 2207, Room 4B-41, Bethesda, MD 20892-2207 OR visit: http://www.jobs.nih.gov and apply to Announcement OD-06-109905 (for Ph.Ds) or OD-06-109915 (for M.Ds).** If you need additional information, please contact **Ms. Teresa Leary at learyte@od.nih.gov or by calling 301-496-1443.**

Applications must be received by close of business April 11, 2006

WWW.NIH.GOV

National Institutes of Health
Office of the Director

Chief NIH Ethics Officer

The Office of the Director, National Institutes of Health (NIH) in Bethesda, Maryland, is seeking a Chief NIH Ethics Officer (CNEO), who will also serve as Director for the NIH Ethics Office. If you are an exceptional candidate with a M.D. and/or Ph.D. and have the vision and skills to oversee and provide strategic direction to NIH activities relating to ethics policy, oversight, and operations for scientific administration, we encourage your application.

To achieve its mission of advancing biomedical and behavioral research to improve the health of the public, the NIH must have the trust of the public that the decisions and activities of the agency and its employees are unbiased. The creation of the new position of CNEO is part of a comprehensive effort to strengthen the program of ethics oversight for NIH employees. As CNEO, you will be responsible for the executive leadership, strategic direction, and oversight of the scientific, clinical, and administrative activities of NIH staff as they relate to ethics policies. This includes: assuring compliance with Federal, departmental, and agency ethics laws, regulations, and policies that apply to the official-duty activities, outside activities, and financial holdings of NIH's 18,000+ employees; overseeing a rigorous program of quality control and risk management including assessing the effectiveness activities of conflict of interest operations the NIH Ethics Office and the application of delegated authority; conducting a comprehensive ethics training program; and developing/maintaining an effective enterprise information technology system. Additional functions include serving as the NIH spokesperson and principal advisor to the Director and Deputy Director, NIH on relevant NIH ethics policy and programs. In addition, you will serve as the NIH Agency Research Integrity Liaison Officer (ARILO) and will be responsible for all matters related to NIH's intramural and extramural research integrity programs to include oversight and coordination of NIH activities related to research misconduct and the promotion of research integrity of NIH intramural and extramural research programs. Understanding the value of scientific expertise for leadership of ethics at NIH, you will have the flexibility to devote up to 25% of your time to conduct or oversee research in an NIH intramural research laboratory or in an appropriate NIH extramural scientific programmatic role.

Salary is commensurate with experience; a full package of benefits is available, including retirement, health, life, long term care insurance, Thrift Savings Plan participation, etc.

Applications for this position will be reviewed by a Search Committee chaired by Dr. Duane Alexander, Director, National Institute of Child Health and Human Development. Applicants may send a Curriculum Vitae to **Teresa Leary, 31 Center Drive - MSC 2207, Room 4B-41, Bethesda, MD 20892-2207 or visit http://www.jobs.nih.gov and apply to Announcement OD-06-109779 (for Ph.Ds) or OD-06-109626 (for M.Ds)**. If you need additional information, please contact **Ms. Teresa Leary at learyte@od.nih.gov or (301) 496-1443**. Applications must be received by close of business **April 11, 2006**.

Postdoctoral Positions
National Institute of Child Health and Human Development

Molecular Neuroscientist
Dax Hoffman, Ph.D., hoffmand@mail.nih.gov
http://neuroscience.nih.gov/Lab.asp?Org_ID=480

Biology of RNA Metabolism in Eukaryotes
Richard Maraia, M.D., maraiar@mail.nih.gov
http://eclipse.nichd.nih.gov/nichd/Maraia/Maraialabpage.html

Nutritional and Behavioral Aspects of Pediatric and Adolescent Obesity
Jack A. Yanovski, M.D., Ph.D., jy15i@nih.gov
http://eclipse.nichd.nih.gov/nichd/deb/ugo/ugo.htm

Molecular Analysis of Pineal Function
David C. Klein, Ph.D., Dr. med. H.c., kleind@mail.nih.gov
http://eclipse.nichd.nih.gov/nichd/ldn/SNE/index.htm

Infant Perception, Cognition, and Cognitive Neuroscience
Marc H. Bornstein, Ph.D., Marc H Bornstein@nih.gov
http://www.cfr.nichd.nih.gov

DNA Replication, Repair, and RNA/DNA Hybrids in Mammalian Cells
Robert J. Crouch, Ph.D., robert_crouch@nih.gov
http://gpp.nih.gov/Researchers/Members/NICHD/RobertCrouch.htm

Chromosome Biology and Mitotic Chromatin
Alex V. Strunnikov, Ph.D., strunnik@mail.nih.gov
http://eclipse.nichd.nih.gov/nichd/test/lgrd/ucsf/index.htm

Signals Regulating T Cell Development
Paul E. Love, M.D., Ph.D., lovep@mail.nih.gov
http://eclipse.nichd.nih.gov/nichd/annualreport/2004/lmgd/scdb.htm

Psychological Development in Middle Childhood/Early Adolescence
Marc H. Bornstein, Ph.D., Marc H Bornstein@nih.gov
http://www.cfr.nichd.nih.gov

Gene Therapy in Animal Models of Menkes Disease
Stephen Kaler, M.D., kalers@mail.nih.gov
http://eclipse.nichd.nih.gov/nichd/annualreport/2004/lcg/upg.htm

Applicants must have less than five years of postdoctoral experience.

NIDDK POSTDOCTORAL FELLOWSHIPS IN SIGNALING PATHWAYS AND DISEASE

Postdoctoral Fellowships are available in the Division of Intramural Research, NIDDK, NIH. The intramural program of the NIH offers an outstanding research environment and has been rated by *The Scientist* as one of the top places for post-doctoral fellows to work. It is located on the main campus of the NIH in Bethesda, Maryland, a 20-minute ride from Washington, D.C. Applications are invited from individuals of the highest caliber who have obtained a Ph.D., or M.D. degree within the last 5 years. Salary and benefits will be commensurate with the experience of the applicant. Interested candidates should send their curriculum vitae, list of publications, a letter stating their interests and the names of three references to the appropriate individual by e-mail or by regular mail. Positions are available in the following areas:

GNAS imprinting and role of the G protein $G_s\alpha$ in metabolic regulation

GNAS is a complex imprinted gene with multiple gene products including the stimulatory G protein $G_s\alpha$ which is the underlying disease gene for several disorders of hormone signaling. Our laboratory studies the mechanisms of *GNAS* and $G_s\alpha$ imprinting in mouse models and in patients with *GNAS* imprinting defects. We are also studying the role of $G_s\alpha$ and other *GNAS* gene products in metabolic regulation using several germline and tissue-specific *Gnas* knockout mouse models. **(Lee S. Weinstein, MD, Bldg. 10 Rm. 8C101, NIDDK/NIH, Bethesda, MD 20892; leew@amb.niddk.nih.gov).**

Molecular Genetics of Signal transduction/tumorigenesis

We have helped discover two genes causing hereditary endocrine cancers (*MEN1* and *HRPT2*). Our efforts are directed at the function of menin, the encoded protein from the *MEN1* tumor suppressor gene. With protein-protein interaction methods, we have identified four menin partners. Our lab also collaborates extensively with other labs in the NIDDK, NCI and NHGRI to study the function of menin in Drosophila and in knockout mice. This position involves creativity and lab expertise with cutting-edge methods. (**Stephen Marx MD, Bldg. 10 Rm. 9C101, NIDDK/NIH, Bethesda, MD 20892; StephenM@intra.niddk.nih.gov**).

Molecular Biology AND Genetics

A post-doctoral position is available to study the mechanism of BRCA1-associated tumorigenesis **(visit http://www.niddk.nih.gov/intram/people/cdeng.htm for details). A strong** background in molecular biology and/or signal transduction is required. **(Chuxia Deng, PhD, Building 10, Room 9N105, 10 Center Drive, Bethesda, MD 20892-1812; ChuxiaD@bdg10.niddk.nih.gov; Tel: (301) 402-7225; Fax: (301) 480-1135).**

NIDDK Immunologist Tenure Track Position

The Diabetes Branch of the National Institute of Diabetes & Digestive & Kidney Diseases, National Institutes of Health, is recruiting a qualified and experienced M.D. or Ph.D. with an interest in pursuing immunological research designed to either elucidate the autoimmune process underlying Type 1 diabetes mellitus (T1DM), develop assays for monitoring that autoimmune process, and/or test immunological interventions to ameliorate the process. The tenure track incumbent would manage his or her own research program but would also be expected to collaborate with existing investigators pursuing basic and clinical research, both within the Diabetes Branch and the broader NIDDK/NIH community.

The Diabetes Branch conducts basic and clinical research designed to better understand the immune-mediated beta cell destruction that underlies T1DM, and/or the target of this autoimmune process - the pancreatic beta cell. Toward that end, Branch investigators have pursued studies ranging from basic biochemical laboratory projects to studies involving a mouse model developed within the Branch, to non-human primate studies, and to clinical and epidemiological studies. While the path toward a T1DM cure remains blocked by several hurdles, the pre-eminent one is an incomplete understanding of the chronic autoimmune process that causes the disease. In addition, investigators interested in overcoming that autoimmune illness are plagued by the lack of assays to follow the autoimmune response and safe therapies for interdicting the autoimmune process.

The Diabetes Branch of NIDDK is located on the main campus of the NIH in Bethesda, Maryland, a suburb of Washington, D.C.

Competititve salary and benefit packages are available. Interested applicants should send a Curriculum Vitae and list of publications, copies of three major publications, a summary of research accomplishments, a plan for future research, and three letters of recommendation by **April 15, 2006** to **Dr. David Harlan, Chair, Search Committee, c/o Ms. Guerdy Toussaint, Diabetes Branch, NIDDK, NIH, Building 10 CRC, Room 5W-5940, 10 Center Drive, Bethesda, MD 20892-1453**.

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NIDDK

Staff Scientist Position
Isotope Ratio Mass Spectrometry Laboratory

The National Institute of Diabetes & Digestive & Kidney Diseases (NIDDK) is establishing a new core facility named the Isotope Ratio Mass Spectrometry Laboratory and invites applications for a mass spectrometrist. The candidate will have the opportunity to set up the mass spectrometry laboratory and then operate and maintain the instrumentation, design and execute experiments in close cooperation with clinical investigators, and participate in the interpretation of the results. The candidate will participate in research meetings and communicate laboratory activities and findings orally and in writing as required. The candidate will work on cutting-edge research in a network of internationally renowned scientists and will enjoy the benefits of joining a supportive scientific community.

Experience in gas chromatography, liquid chromatography and isotope ratio mass spectrometry techniques is required. Prior knowledge of the theoretical and practical matters related to preparation and analysis of biological samples for isotopically labeled glucose, lipid and doubly labeled water experiments is preferred.

This core facility is located on the main NIH campus in Bethesda, Maryland, a suburb of Washington, D.C. Clinical investigators comprising the major points of contact for this position perform patient-oriented research in a number of areas of endocrinology and metabolism with a special emphasis on studies of obesity. Salary and benefits will be commensurate with the experience of the applicant.

The successful candidate will have a Masters or Ph.D. in analytical chemistry, biochemistry or a related field, with knowledge and interest in the following: liquid/gas chromatography and isotope ratio mass spectrometry. Interested candidates should submit a curriculum vitae, bibliography, a summary of accomplishments and arrange for three letters of reference to be sent to:

Ms. Jackie Collier, Office of the Director, Division of Intramural Research, NIDDK, National Institutes of Health, Bldg. 10, Room 9N222, MSC 1818, 9000 Rockville Pike, Bethesda, MD 20892.

NIH SCIENTIFIC REVIEW ADMINISTRATOR
(Health Scientist Administrator)
Vacancy: CSR-06-111051

Center for Scientific Review

We are seeking a qualified scientist, with doctorate level training and independent research experience in the neural basis of behavior to join a team of Scientific Review Administrators (SRAs) to help shape the future of scientific review. The incumbent will be responsible for the initial administrative and scientific review of NIH neuroscience research grant applications and will possess an M.D. or Ph.D. degree (or have equivalent training and experience), have independent research experience and a strong publication record. A broad knowledge of neuroscience is desirable, with a specific emphasis on the neural basis of behavior such as motivation and emotion. The position is in the Integrative, Functional, and Cognitive Neuroscience (IFCN) Integrated Review Group (IRG). This IRG is responsible for administering the review of a wide range of neuroscience research aimed at furthering our understanding of how the nervous system is organized and functions at an integrative, systems level. For additional information on the IRG please see our web site, at: http://cms.csr.nih.gov/PeerReviewMeetings/CSRIRGDescription/IFCNIRG/

Salary is commensurate with research experience and accomplishments, and a full Civil Service package of benefits (including retirement, health, life and long-term care insurance, Thrift Savings Plan participation, etc.) is available.

For additional information on this position, and for instructions on submitting your application, please see our website, at: http://jobsearch.usajobs.opm.gov/ listed under vacancy announcement number **CSR-06-111051**.

The closing date for this position is **March 17, 2006.**

Associate Director for Basic Research
UNMC Eppley Cancer Center

The University of Nebraska Medical Center (UNMC) Eppley Cancer Center, a National Cancer Institute-designated Cancer Center, seeks outstanding candidates for the position of Associate Director for Basic Research. This Associate Director position will include a tenured appointment with academic rank commensurate with experience.

The successful applicant will be responsible for the overall direction and development of the Cancer Center's basic research programs. Responsibilities include maintaining an independent research program and fostering the continued development of basic research programs and interdisciplinary collaborations. This person will advise the Director on promising areas of research, provide direction to faculty members in pursuing research objectives, and be responsible for the Cancer Center's basic research shared facilities.

The UNMC Eppley Cancer Center is in a dynamic growth phase and committed to expansion of all its research programs. Growth in the cancer research programs is aided by generous support from the Nebraska Tobacco Settlement Biomedical Research Funds. With a strong commitment of both public and private funds, UNMC has made strategic investments in its research infrastructure with the addition of the Durham Research Center and the Lied Transplant Center which provide state of the art laboratory and clinical space for cancer research. UNMC plans to add another 242,000 square foot research building next year which will provide for continued growth of the Cancer Center.

Applicants should have a history of significant peer-reviewed funding, strong interpersonal and communication skills, and evidence of successful scientific collaborations. Experience in a leadership position within an NCI-designated Cancer Center is preferred. The position includes a generous start-up package and a primary appointment in the Eppley Institute for Research in Cancer and Allied Diseases.

Candidates should have a Ph.D. and/or M.D. degree. Applicants can apply online to position **# 1015** at **https://jobs.unmc.edu**. Additional information can be found at **http://www.unmc.edu/cancercenter/**. Candidates should forward a minimum of 3 letters of reference to:

Kenneth H. Cowan, M.D., Ph.D.
Director, Eppley Institute for Research in Cancer
Director, UNMC Eppley Cancer Center
University of Nebraska Medical Center
986805 Nebraska Medical Center
Omaha, NE 68198-6805
kcowan@unmc.edu

The University of Nebraska Medical Center is an Equal Opportunity Employer.

454 LIFE SCIENCES

454 Life Sciences, a 66% majority-owned subsidiary of CuraGen Corporation (Nasdaq: CRGN), has developed and is commercializing a revolutionary novel sequencing instrument. Our customers currently generate over 20 million nucleotide bases per five-hour run, totaling more than 100 times the capacity of instruments using the current macro-scale technology. Check out our technology at **http://www.454.com**.

We are looking for talented employees with can-do attitude that want to be part of the next generation sequencing technology revolution. It would be preferable if you have industrial experience from the life science industry and in particular have hands on product development experience. Prior experience with sequencing technology would be great but is not a requirement.

We have the following position openings:

- Research Scientists
- Research Associates
- Software Engineers
- Software/Informatics
- Senior Technical Product Manager
- Inside Sales Manager

If you would like to apply for any of these positions and come help us change the world by developing cutting edge technologies that enable massive scale genome analysis at the speed of light, please email your cover letter and resume to **careers@454.com**.

Featured Employers

Search **ScienceCareers.org** for job postings from these employers. Listings updated three times a week.

Abbott Laboratories
www.abbott.com

Genentech
www.gene.com

Kelly Scientific Resources
www.kellyscientific.com

Pfizer, Inc.
www.pfizer.com

Pierce Biotechnology, Inc.
www.piercenet.com

Scios
www.sciosinc.com

If you would like to be a featured employer, call 202-326-6543.

ScienceCareers.org
We know science AAAS

GFZ POTSDAM

in der Helmholtz-Gemeinschaft

Das **GeoForschungsZentrum Potsdam (GFZ Potsdam)** ist eine von der Bundesrepublik Deutschland - vertreten durch das Bundesministerium für Bildung und Forschung (BMBF) - und dem Land Brandenburg gemeinsam finanzierte Großforschungseinrichtung in der Helmholtz-Gemeinschaft Deutscher Forschungszentren (HGF).

Forschungsgegenstand des GFZ Potsdam ist das System Erde. Die FuE-Aktivitäten sind im Helmholtz-Forschungsbereich "Erde und Umwelt" angesiedelt und konzentrieren sich u. a. auf die Themenfelder: Globale Prozesse und Geomonitoring, Geodynamik und natürliche Ressourcen, Georisiken und Katastrophenvorsorge sowie Geoengineering und Nutzung des Untergrunds. Für die eigenen Forschungsarbeiten und die Gemeinschaftsforschung, die mit Universitäten und anderen Partnern in nationaler und internationaler Vernetzung durchgeführt wird, steht eine umfangreiche wissenschaftlich-technische Infrastruktur (u. a. Gerätepools) zur Verfügung. Das GFZ Potsdam hat zur Zeit ein jährliches Gesamtbudget von ca. 65 Mio. € und beschäftigt etwa 650 Mitarbeiter/Mitarbeiterinnen. Die Leitung besteht aus einem Wissenschaftlichen und einem Administrativen Vorstand. Zum **1. Juni 2007** ist im GFZ Potsdam die Stelle des

Wissenschaftlichen Vorstands, zugleich Sprecher/in des Vorstands,

neu zu besetzen.

Der Wissenschaftliche Vorstand ist Repräsentant/in des Forschungszentrums. In den Aufgabenbereich fallen insbesondere die wissenschaftliche Entwicklung und der Ausbau der Einrichtung im nationalen und internationalen Wissenschaftsraum sowie Forschungsplanung und Erfolgskontrolle in Zusammenhang mit den Organen und Aufsichtsgremien.

Die Aufgaben verlangen eine Persönlichkeit, die über eine hervorragende Qualifikation in der naturwissenschaftlichen Grundlagenforschung und über Erfahrungen in leitenden Funktionen des Forschungsmanagements verfügt. Strategisches Gestaltungsvermögen muss sich in der Person mit praktischer Überzeugungskraft sowie Integrations- und Durchsetzungsfähigkeit verbinden.

Die Bestellung erfolgt für die Dauer von fünf Jahren. Wiederbestellung ist möglich. Die Vergütung orientiert sich an dem Vergütungsrahmen für herausgehobene Hochschulprofessoren.

Die Mitglieder der Helmholtz-Gemeinschaft haben sich die Förderung von Frauen in Führungspositionen zum Ziel gesetzt. Bewerbungen von Frauen werden daher begrüßt.

Bitte richten Sie Ihre Bewerbung mit entsprechenden Unterlagen bis zum **15. März 2006** an:

Ministerialdirektor Reinhard Junker, Vorsitzender des Kuratoriums des GFZ
c/o Bundesministerium für Bildung und Forschung, D-53170 Bonn

Reinhard Junker (Vorsitzender des Kuratoriums)

NEUROSCIENCE FELLOWS

Applications are invited for 2-3 Neuroscience research fellowships at the Albert Einstein College of Medicine. These fellowships provide an exceptional opportunity for MDs to engage in full time research for 12 months under the guidance of leading experts in the field of neuroscience. The fellowships provide a base stipend of $65,000 per annum, with the possibility of renewal for a second year on a competitive basis.

Requirements: Applicants must be US citizens or permanent resident MDs who have passed USMLE parts I and II, or ECFMG. Preference would be given to applicants with prior research experience, and those who foresee a significant research component in their career.

Application procedure: Applicants should identify and secure the support of one or more potential mentors from the neuroscience and psychiatry faculty at AECOM. The list of faculty with primary and secondary appointments in the Neuroscience Dept may be found at: **www.kennedy.aecom.yu.edu/neuroscience/.** 2 letters of recommendation, a 2 page description of career goals and plans for the fellowship, and a letter of support from the potential neuroscience faculty mentor(s) should be sent by March 15, 2006 to: **Dr. Donald S. Faber, Dept of Neuroscience, Albert Einstein College of Medicine, 1410 Pelham Parkway South, Bronx, NY 10461**. The successful applicants will be notified by April 15, 2006. EOE

ALBERT EINSTEIN COLLEGE OF MEDICINE
Advancing science, building careers

Department of Health and Human Services
National Institutes of Health
National Institute on Aging

The National Institute on Aging, a major research component of the National Institutes of Health (NIH) and the Department of Health and Human Services (DHHS) is recruiting for **four post-doctoral fellows** in the Laboratory of Genetics, Intramural Research Program (IRP):

1) with a background in cell based screens or imaging studies to work in the Image Informatics and Computational Biology Unit (IICBU), for high-throughput automated visual screening of RNAi libraries. The interdisciplinary group has developed image classification algorithms based on machine learning techniques, and we would like to apply these to the systematic reconstruction of genetic pathways. For additional information on this research, please go to: **(http://www.grc.nia.nih.gov/branches/lg/iicbu/iicbu.htm)**. Applicants should send the curriculum vitae, via email to Dr. Ilya Goldberg at, **goldbergil@grc.nia.nih.gov**.

2) with a background in biochemistry to work in the Transcription Regulation and Remodeling Section (TRRS), on purification of multi-protein complexes and analysis of their structures and functions **(http://www.grc.nia.nih.gov/branches/lg/trru/trru.htm)**. Projects include studies of chromatin-remodeling mechanisms (*G&D 19:1662-7*), DNA damage response, and human genomic instability diseases (*Nat. Genet. 35:165-170; 37: 958-63*). Applicants should send the curriculum vitae, via email to Dr. Weidong Wang, **wangw@grc.nia.nih.gov**

3) with a background in mouse development to work in the Developmental Genomics and Aging Section, to conduct the study of preimplantation mouse development (*Dev. Cell 6: 117-131, 2004*) and embryonic stem cells (*PLoS Biol. 1: 410-419, 2003*). The work utilizes embryogenomics approaches (*Trends Biotechnol. 19: 511-518, 2001*) and focuses on the identification and characterization of genes that are critical for the maintenance of pluripotency and/or for early commitment to different cell lineages. Applicants should send the curriculum vitae via email to Dr. Minoru Ko, **kom@grc.nia.nih.gov**.

4) with a background in molecular genetics to work in the Human Genetics Section, on the determination of skin appendage formation in vitro, based on signaling pathways operating with the EDA (ectodysplasin) TNF-ligand (*Hum. Molec. Genet. 11:1763-1773; Hum. Molec. Genet. 12: 2931-2940*). The aims include the understanding of how hair follicles form, as a model system for both development and possible regeneration. Approaches include histology, keratinocyte cell differentiation, and immunocytochemistry, as well as a range of genomic and physiological techniques. Applicants should send the curriculum vitae, via email to Dr. David Schlessinger, **schlessingerd@grc.nia.nih.gov.**

The successful individuals will possess an M.D. or Ph.D. degree in biochemistry, molecular genetics or a related field, with no more than five years of Post Doctoral research experience. Salary is commensurate with research experience and accomplishments.

DHHS and NIH are Equal Opportunity Employers

Science Careers Forum

Entrepreneur's Week
27 February – 3 March

Science Careers welcomes two distinguished guest hosts to the Forum. These experts will offer insights into their entrepreneurial careers and the different approaches they took.

Dr. Joseph M. DeSimone
Professor of Chemistry
University of North Carolina

Dr. Avi Spier
Director of Business Development
Genomics Institute of the
Novartis Research Foundation

Visit ScienceCareers.org and click on Career Forum between 27 February and 3 March.

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Office of Research and Development
National Center for
Environmental Assessment (NCEA)

Supv. Biologist/Toxicologist/Health Scientist/Physical Scientist/Mathematical Statistician
Ez hire Announcement #RTP-DE-2006-0048 or RTP-MP-2006-0080

The U.S. Environmental Protection Agency is seeking highly qualified applicants for two Branch Chief positions with the National Center for Environmental Assessment (**http://cfpub.epa.gov/ncea/**) which are located in Cincinnati, Ohio. Duties include supervision and leadership of an interdisciplinary team of scientists conducting high-profile human health and ecological assessments and developing cutting-edge risk assessment methods, with emphasis on water quality and hazardous waste.

Excellent benefits: The selected candidate will be eligible for a full benefits package, including paid relocation, health insurance, life insurance, retirement, and vacation and sick leave. This is a permanent, full time position. U.S. citizenship is required.

Salary Range: The salary range is $91,080 to $139,275 (GS 14/15) per year, commensurate with qualifications.

Qualifications: A bachelor's degree (or higher) is required. Desirable applicants will have an advanced degree and demonstrated experience in conducting research and leading research teams in environmental health, toxicology, biology, physical science, mathematical statistics, or a related field.

How to Apply: Applicants should apply through Ezhire at **http://www.epa.gov/ezhire** Select apply for jobs. If you are already registered in Ezhire@EPA system, access the vacancy announcement through Registered Users. Otherwise, select New Users and complete the registration process. The vacancy announcement will be open through March 13, 2006. Application materials must be submitted with 48 hours from the closing date of the announcement. You need to submit the additional documentation described in the full text vacancy. Questions regarding this vacancy may be directed to **Joann Kelleher, Human Resources Management Division** at **kelleher.joann@epa.gov**.

The US EPA is an Equal Opportunity Employer.

The Department of Pathology
at The University of Chicago

POSTDOCTORAL SCHOLAR

The laboratory of H. Rosie Xing at The University of Chicago, Department of Pathology, has an opening for a Postdoctoral Scholar with a strong record of productivity. Our laboratory studies molecular mechanisms that govern gain-of-function Ras signaling of human oncogenesis via activated EGFRs, or oncogenic activation of Ras, and translates this information to develop therapeutic strategies for cancer treatment (*Nat. Med.,* 19: 1267, 2003; *Cancer Res.,* 63: 4232, 2003). We are also interested in exploring molecular mechanisms underlying cellular sensitivity to ionizing radiation and to devise strategies to regulate radiosensitivity by manipulating signal transduction events.

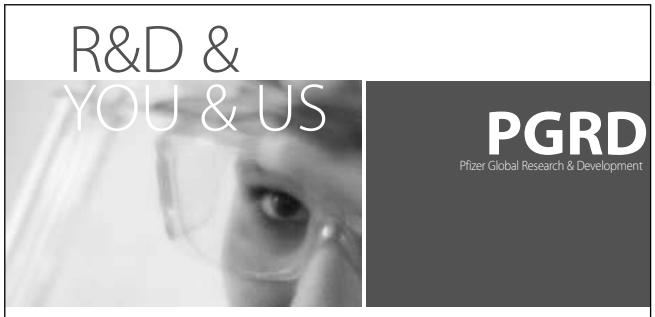
The successful applicant will have a Ph.D., M.D. or M.D./Ph.D. with experience and technical skills in cancer biology and molecular biology. Candidates with experience of xenograft models of human cancer or DNA damage repair are highly desirable. Salary and benefits are competitive and commensurate with experience.

Interested applicants should send a cover letter along with a curriculum vitae and the names of three to five references to: **hxing@bsd.uchicago.edu** or to:

H. Rosie Xing, Ph.D., Assistant Professor
Department of Pathology and Radiation Oncology
University of Chicago
5841 S. Maryland Ave. – MC 1089
Chicago, Illinois 60637

THE UNIVERSITY OF CHICAGO

The University of Chicago is an equal opportunity/affirmative action employer and welcomes applications from women and minorities.



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We have Postdoctoral Fellow opportunities at our **St. Louis** facility in the following areas:

Cardiovascular

You'll quantify the calcium signal and degree of myocyte contraction in dissociated rat cardiac cells as well as the ion currents involved in producing cardiac cell action potential. Requires an expertise in imaging with experience in recording from isolated cardiac and smooth muscle cells. Extensive knowledge of vascular smooth muscle cells and in vivo pharmacology is essential. **Req # 052317**

Pain Pharmacology

Use molecular, cellular and in vivo models to study the mechanisms and pathways related to pain. You'll need a biomedical background with training in animal models of pain and experience in research associated with pain, neuroscience or lipid biology. **Req# 052320**

Chemistry

Investigate the use of automated synthesis/flow-chemistry devices for the development of new synthetic methods, preparation of novel compounds and synthesis of compound libraries. Our ideal candidate has a demonstrated understanding of organic chemistry and synthetic methods along with experience in analytical instrumentation. **Req # 052371**

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POSITIONS OPEN

University Of Nebraska Lincoln

NEBRASKA CENTER FOR VIROLOGY

RESEARCH ASSISTANT PROFESSOR in molecular virology to participate in established research program of **Dr. Charles Wood** on HIV and AIDS-associated diseases including Kaposi's Sarcoma. Provide management of ongoing research projects and supervise research staff in his laboratory while developing independent research associated with the Nebraska Center for Virology **(website: http://www.unl.edu/virologycenter)** through the University of Nebraska, Lincoln School of Biological Sciences. Candidates should hold Ph.D. and/or M.D. and relevant postdoctoral research experience.

Review begins April 1, 2006. To be considered for this position, apply at **website: http://employment.unl.edu.**

Dr. Charles Wood, Director
Nebraska Center for Virology
E249 Beadle Center
Lincoln, NE 68588-0666
E-mail: cwood1@unl.edu

The University of Nebraska is committed to a pluralistic campus community through Affirmative Action and Equal Opportunity. We assure reasonable accommodation under the Americans with Disabilities Act; contact ***J. Walker*** *at* ***telephone: 402-472-4560*** *for assistance.*

FACULTY POSITION

Applications are invited for a tenure-track position at the **ASSISTANT** or **ASSOCIATE PROFESSOR** level in the Department of Physiology and Biophysics, University of Miami Miller School of Medicine. (In exceptional cases, an appointment at tenured full professor will be considered.) We seek outstanding candidates with demonstrated research interests in the neurosciences and/or physiology and biophysics that complement the existing research focuses in the department **(website: http://chroma.med.miami.edu/physiol/faculty.htm).** Candidates should have a Ph.D. and/or M.D. degree in a relevant field as well as postdoctoral training and publications in highly rated journals. The successful candidate will be expected to conduct an active, independent, funded research program, and to contribute teaching with excellence in medical and graduate courses in neuroscience and physiology. Send paper copies of: letter of application, curriculum vitae, statements of research and teaching interests, and copies of three publications to: **Dr. Karl Magleby, Chair, Department of Physiology and Biophysics, R-430, University of Miami Miller School of Medicine, 1600 N.W. 10th Avenue, Miami, FL 33136.** Letters of recommendation from three references should be sent directly to the same address. Review of applications will commence when received and will continue until the position is filled. *The University of Miami is an Equal Opportunity/Affirmative Action Employer.*

NATIONAL UNIVERSITY OF SINGAPORE
Department of Chemical and Biomolecular Engineering

The Department of Chemical and Biomolecular Engineering at National University of Singapore invites applications for **TENURE-TRACK FACULTY** positions at all levels. The Department is one of the largest internationally with excellent in-house infrastructure for experimental and computational research. A Ph.D. in chemical engineering or related areas and a strong research record with excellent publications are required. Please refer to **website: http://www.chbe.nus.edu.sg/** for more information on the areas of interest and for application details. Applicants should send full curriculum vitae (including key publications), a detailed research plan, a statement of teaching interest, and a list of names of at least three references to: **Professor Raj Rajagopalan, Head of Department (Attention: Ms. Nancy Chia, e-mail: nancychia@nus.edu.sg).**

POSITIONS OPEN

CASE WESTERN RESERVE UNIVERSITY

CARL ASSEFF ENDOWED CHAIR IN VISION RESEARCH

The Department of Ophthalmology at Case Western Reserve University and University Hospitals of Cleveland is actively seeking a senior or mid-level investigator for the Carl Asseff Endowed Chair in vision research. Candidates are expected to have a strong record of external funding and peer-reviewed publications. The basic science research of the Department is relocating to newly renovated space, which will be a part of the Visual Sciences Research Center (VSRC). The VSRC **(website: http://www.case.edu/med/vsrc)** includes over twenty faculty members from several basic science departments, and has a P30 Core Grant and a T32 Training Grant on the visual sciences from the National Eye Institute. VSRC investigators have interests in cataract formation, diabetic retinopathy, eye muscle disorders, genetics, ocular immunology, and inflammation, and the retinal degenerations, and have access to new expertise in the Center for Proteomics and functional imaging in the Small Animal Imaging Center. The candidate will benefit from a collaborative environment and will have a secondary appointment in a basic science department and will be expected to participate in graduate education. Applicants should submit their curriculum vitae and research interests as a single PDF file to **e-mail: ophthalmology@case.edu.** *Case Western Reserve University and University Hospitals of Cleveland are Equal Opportunity, Affirmative Action Employers.*

TENURE-TRACK FACULTY POSITION, BIOCHEMISTRY. Applications are invited to fill a tenure-track position at the rank of Assistant Professor in biochemistry to begin in fall 2006. Candidates must demonstrate potential for establishing a vigorous and externally funded research program and must also exhibit a commitment to excellence in graduate and undergraduate teaching. Applicants must have a Ph.D. degree and postdoctoral research experience. Research interests in all areas of biochemistry will be considered. Teaching responsibilities will be in biochemistry and related areas. Applicants should send: (i) curriculum vitae; (ii) a summary of research experience indicating the applicant's most important contributions; (iii) detailed plans for future research; (iv) a statement of teaching philosophy and interests; and (v) copies of undergraduate and graduate transcripts. Applicants should arrange for three letters of recommendation to be sent directly to the Department Chair. Application materials and letters of recommendation should be sent to: **Dr. Roger B. Gregory, Chair, Department of Chemistry, Kent State University, P.O. Box 5190, Kent, Ohio 44242-0001.** Review of applicants will begin immediately. Applications will continue to be accepted until the position is filled. Position will be filled pending final budgetary approval. All documents submitted to Kent State University for employment opportunities are public records and subject to disclosure under the Ohio Public Records Law. *Kent State University is an Equal Opportunity, Affirmative Action Employer.*

TWO POSTDOCTORAL POSITIONS are available in the **Department of Biomedical Engineering** at **Georgia Tech and Emory University** for experienced Scientists or Engineers in the area of living cell gene detection. Preference will be given to highly motivated Ph.D.s who have a strong background in biochemistry/organic synthesis, fluorescence microscopy, or functional genomics, and with excellent written and verbal communication skills. Interested candidates please submit your curriculum vitae with a list of three references to **e-mail: gang.bao@bme.gatech.edu.**

POSITIONS OPEN

DIRECTOR, DIVISION OF UNDERGRADUATE EDUCATION
National Science Foundation, Arlington, Virginia

NSF's Directorate for Education and Human Resources seeks candidates for the position of Director, Division of Undergraduate Education (DUE). The Division serves as a focal point for NSF's agency-wide commitment in promoting excellence in undergraduate science, technology, engineering, and mathematics (STEM) education for all students. Information about the Division's activities may be found at **website: http://www.nsf.gov/div/index.jsp?org=DUE.**

Appointment to this Senior Executive Service position may be on a career basis, on a one-to-three year limited term basis, or by assignment under the Intergovernmental Personnel Act (IPA) provisions.

Announcement S20060048, with position requirements and application procedures are posted on NSF's Home Page at **website: http://www.nsf.gov/about/career_opps/.**

Applicants may also obtain the announcements by contacting Executive Personnel Staff at **telephone: 703-292-8755** (Hearing impaired individuals may call **TDD 703-292-8044**). Applications must be received by March 24, 2006. *NSF is an Equal Opportunity Employer.*

ASSISTANT/ASSOCIATE PROFESSOR
Cancer Biology

The University of Colorado Comprehensive Cancer Center and the Department of Pathology at the University of Colorado School of Medicine invite applications for a full-time, tenure-track position in Cancer Biology at the Assistant or Associate Professor level, commensurate with experience and accomplishments. Applicants should have a Ph.D., M.D. or M.D./Ph.D. degree, postdoctoral research experience in cancer biology or a related field, and an exceptional record of research accomplishments. Individuals with experience in the area of cancer biology including malignant transformation, cell proliferation, signal transduction, cell motility and migration, metastasis, and apoptosis are especially encouraged to apply. The successful applicant will be expected to develop a vigorous externally funded research program and contribute to the teaching mission at the School of Medicine and the Graduate School.

Applicants should submit curriculum vitae, brief description of research interests, and arrange to have three letters of reference forwarded to the address listed below by March 15, 2006:

Steven M. Anderson, Ph.D.
University of Colorado UCDHSC
Department of Pathology, Mailstop 8104
P.O. Box 6511
Aurora, CO 80045
E-mail: steve.anderson@uchsc.edu

The University of Colorado is committed to diversity and equality in education and employment.

Find out about jobs before you get your issue. Sign up for customized e-mail notification of jobs at **website: http://www.sciencecareers.org** by clicking on Job Alerts. You can also post your resume (open or confidentially) and check how many employers have viewed your resume at your own convenience.

POSITIONS OPEN

The **U.S. CIVILIAN RESEARCH AND DEVELOPMENT FOUNDATION (CRDF)**, an international nonprofit supporting scientific collaboration between American and foreign Scientists, seeks a dynamic leader and team builder for the position of **VICE PRESIDENT FOR PROGRAMS.**

The successful candidate should possess extensive (minimum of ten years) managerial and grant making or business partnership experience, preferably in the nonprofit or private sectors. Substantial knowledge of international science and technology research and business is essential; experience working internationally, particularly in Eurasia or the Middle East, is highly desirable. He/she must demonstrate through professional experience and specific accomplishments an entrepreneurial mindset, as well as outstanding diplomatic, communications, and interpersonal skills. Overseas travel (an average of six trips annually) is required.

Responsibilities include supervising five grant-making divisions and a staff of over 40 people; representing the CRDF in negotiations with government and private organizations in the United States and abroad; responding to requests for information from U.S. funding organizations, including the U.S. government and private foundations; and coordinating with CRDF financial, administrative and development departments. The Vice President for Programs reports to the CEO/President and is responsible for coordinating program planning and execution with the CEO/President. The Vice President for Programs serves on the CRDF Senior Management Committee and contributes to CRDF strategic planning. He/she interacts regularly with the CRDF Board of Directors and is responsible for coordinating the agenda and preparing background materials for Board Program Committee meetings.

A Master's degree or higher, or an equivalent combination of education and experience is required, along with 10 years of international science and technology experience and at least five years of supervisory management experience. *U.S. citizenship is required.*

Please submit curriculum vitae and letter by March 15, 2006 to:

U.S. Civilian Research and Development Foundation
Attention: Vice President, Programs
1530 Wilson Boulevard, 3rd Floor
Arlington, VA 22209
Fax: 703-526-9721; e-mail: hr@crdf.org

UNIVERSITY OF ILLINOIS AT CHICAGO, DEPARTMENT OF CHEMISTRY is soliciting applications for a **DIRECTOR OF NUCLEAR MAGNETIC RESONANCE (NMR) FACILITIES.** A Ph.D. or advanced degree with extensive experience in the use and applications of Nuclear Magnetic Resonance is required. This position will direct the operation of the NMR laboratories that serve the Department of Chemistry and will be responsible for maintaining and upgrading the NMR instruments, training graduate students in the use of these instruments, implementing new and advanced experiments and providing technical guidance to users. In addition to supervising student assistants in their NMR service duties, the successful candidate will assist in the development of proposals for new instruments. Please submit application, including curriculum vitae, and letters of reference from three individuals who are familiar with the candidate's work by March 28, 2006, to: **Dr. Duncan Wardrop, Chair, Nuclear Magnetic Resonance Director Search, Department of Chemistry (M/C 111), The University of Illinois at Chicago, 845 W. Taylor Street, Chicago, Illinois 60607-7061.** *University of Illinois at Chicago is an Affirmative Action/Equal Opportunity Employer.*

POSITIONS OPEN

Chicago's Jesuit University
LOYOLA UNIVERSITY CHICAGO
Stritch School of Medicine

TENURE-TRACK TENURE-TRACK ASSISTANT/ASSOCIATE PROFESSORS

The Department of Pharmacology at Loyola University Chicago, Stritch School of Medicine is recruiting tenure-track Assistant/Associate Professors to establish their own independent research as well as interact with existing faculty within the Department and the Cardiovascular, Neuroscience, and Oncology Institutes. Candidates whose research focuses on the dissection of fundamental mechanisms for clinical/therapeutic applications are highly encouraged to apply. Generous startup funds and laboratory space are available. For more Departmental information visit **website: http://www.luhs.org/depts/pharmacology/index.html.** Applicants should have a Ph.D. and/or M.D. degree, and be committed to excellence in research and teaching of Pharmacology. Applications should include curriculum vitae and a research interest statement. Three letters of reference to support the candidacy should be sent separately. Address all correspondence to: **Dr. Tarun B. Patel, Chair, Department of Pharmacology, Loyola University Chicago, Stritch School of Medicine, 2160 S. First Avenue, Maywood, IL 60153.** (No electronic applications.) *Equal Employment Opportunity/Affirmative Action Employer.*

BONE AND SUTURE BIOLOGY RESEARCH SCIENTIST (DOCTORAL LEVEL)

We are interested in establishing a multidisciplinary Center for the developmental biology of the craniofacial skeleton and its structure-function relationships. The Center will focus on normal and abnormal physiologic processes in craniofacial biology, using models which may translate to the care of children with craniofacial anomalies. The Center will have joint sponsorship by the Departments of Plastic Surgery and Physiology. We also anticipate collaboration with the Children's Research Institute. We are currently seeking a qualified Scientist to assume a central role in these activities. Qualifications would include a Ph.D. in molecular or developmental biology with specific experience and skills in the area of transgenic research. This position is a full-time academic faculty post, with rank commensurate with experience. The overarching goal is to advance the understanding of craniofacial biology at a molecular and cellular level to elucidate specific pathologic processes and translate these findings into improved or even novel therapeutic intervention. Applicants should anticipate involvement in collaborative and NIH-supported programmatic research that would include both clinical and basic Scientists with common interests in these problems. Competitive salary support is provided for the first two years, but contribution by the candidate is expected in the form of independent, peer-reviewed grant support over time. Interested candidates should submit their curriculum vitae and three letters of reference to: **Arun K. Gosain, M.D., Center for Craniofacial Disorders, Children's Hospital of Wisconsin, P.O. Box 1997, M.S. C340, Milwaukee, WI 53201.**

ScienceCareers.org
We know science AAAS

Find out about jobs before you get your issue. Sign up for customized e-mail notification of jobs at **website: http://www.sciencecareers.org** by clicking on Job Alerts. You can also post your resume (open or confidentially) and check how many employers have viewed your resume at your own convenience.

EUROPEAN SCIENCE FOUNDATION

Opportunities for Europe-wide Collaborations

The European Science Foundation (ESF) is the European association of 78 national research organisations in 30 countries, with offices in Strasbourg and Brussels, devoted to scientific research. The ESF covers all scientific domains: physical and engineering sciences; life, earth and environmental sciences; medical sciences; humanities; and social sciences.

The mission of ESF is to provide a common European platform for its Member Organisations (MOs) in order to advance research and explore new directions for research at the European level.

These goals will be reached by three fundamental approaches:

SCIENCE STRATEGY
providing high level and high quality foresight and advice on science, research infrastructure and science policy issues of European significance. The ESF achieves this with **Forward Looks**, a major contribution towards common MO strategies for European Research, and **Exploratory Workshops**.

SCIENCE SYNERGY
stimulating cooperation between researchers and Member Organisations, in order to explore new directions in research throughout Europe. This is achieved through **ESF Research Networking Programmes; EUROCORES and ESF Research Conferences.**

SCIENCE MANAGEMENT
underlining the vital organisational role that ESF can play in a European setting, via the **EURYI** awards; **COST** (European CO-operation in the Field of Scientific and Technical Research) and **Eurobiofund**.

In 2006 ESF announces the following Calls for Proposals

Proposals for ESF Exploratory Workshops

Small, interactive group sessions aiming to open up new directions in research or to explore emerging research fields. Proposals should demonstrate the potential to generate follow-up research activities.

Award will be to a max. value of €15 000
Submission open from: **1st March 2006**
Deadline for submitting proposals: **2nd May 2006**
Awarded workshops should take place during 2007
For further information: **www.esf.org/workshops**

EUROCORES (**Euro**pean Science Foundation **Co**llaborative **Re**search)

The scheme provides a framework to bring together national research funding organisations to support European research. **THEMES:** The EUROCORES Theme Call is an important step to identify new areas for European collaborative research across all scientific fields. This creates future funding opportunities by developing the themes for new EUROCORES programmes.

Submission open from: **March 2006 (expected)**
Deadline for submitting proposals: **1st June 2006**

PROJECTS: Each year a call for Collaborative Research Projects (CRPs) is announced, based on EUROCORES themes selected the previous year.

For further information: **www.esf.org/eurocores**

Proposals for ESF Research Networking Programmes

Long term networking activities bringing together nationally funded research groups, to address a major scientific or research infrastructure issue. Proposals must show the potential to be carried out at the European level.

Award will be to a max. value of up to €120 000 annually, normally 4-5 years
Submission open from: **1st July 2006**
Deadline for submitting proposals: **30th October 2006**
New Programmes should be launched from **1st January 2008**
For further information: **www.esf.org/programmes**

Applications for EURYI Awards

These awards enable outstanding young scientists in any area of scientific research, from any country in the world, to create their own research teams at European universities and other research institutions. EURYI is a joint initiative of Eurohorcs and ESF. The 4th EURYI Call is expected to open later this year.

Award will be to a max. value of up to €1 250 000 over a five-year period
Submission open from: **1st September 2006 (expected)**
Deadline for submitting proposals: **30th November 2006**
For further information: **www.esf.org/euryi**

Also during 2006

ESF Research Conferences will be announcing a separate call in the spring.
For further information: **www.esf.org/conferences**

COST (European **CO**-operation in the Field of **S**cientific and **T**echnical Research) will be announcing their first open call this year. For further information: **www.cost.esf.org**

COST and the management and networking of EURYI and EUROCORES are supported by grants from the European Commission.

YYePG Proudly Presents, Thx for Support

For information on all ESF activities, please go to www.esf.org

POSITIONS OPEN

POSTDOCTORAL RESEARCH SCIENTIST
Mouse Model of HIV-1 Infection

Postdoctoral Research Scientist positions are available at the Molecular Virology Division, Columbia University Medical Center/St. Luke's-Roosevelt Hospital Center for research in a recently developed mouse model of HIV-1 infection and pathogenesis. Ongoing work with this model includes studies on HIV-1 replication in mouse cells in vitro and in vivo, elucidation of mechanisms of HIV-1-mediated neuropathogenesis, and investigations of immune responses to HIV-1 in mice, with potential applications to HIV-1 vaccine research. Postdoctoral candidates should have experience in one or more of the research areas indicated above. The candidates for Instructor must have extensive experience in HIV-1 molecular biology and a solid background in cellular and functional aspects of HIV-1 interaction with brain and immune system cells. Prior experience in animal work and neuropathology would be valuable. Ph.D., M.D., or equivalent degree is required.

Send resume and names of three references to **Dr. David J. Volsky** at **e-mail: djv4@columbia.edu.**

Columbia University Medical Center takes Affirmative Action to insure Equal Opportunity.

UNIVERSITY OF PENNSYLVANIA

POSTDOCTORAL POSITION in signal transduction to examine the role of prolyl hydroxylases in the regulation of Hypoxia Inducible Factor (**Yu et al.,** *PNAS* **98:** 9630, 2001; **Percy et al.,** *PNAS* **103:** 654, 2006,). Experience in molecular biology required, and with mouse models preferred. Send curriculum vitae and the names of three references to: **Dr. Frank Lee, Department of Pathology and Lab Medicine, University of Pennsylvania School of Medicine, 605 Stellar Chance Labs, Philadelphia, PA 19104. E-mail: franklee@mail.med.upenn.edu.** *Affirmative Action/Equal Opportunity Employer.*

POSITIONS OPEN

ENCYSIVE PHARMACEUTICALS

SENIOR SCIENTIST, GPCR BIOLOGIST

Encysive Pharmaceuticals is a rapidly growing pharmaceutical company located in the heart of the Medical Center in Houston, Texas. We are seeking a highly motivated, creative Scientist to complement the company's existing G protein-coupled receptor (GPCR) programs. A Ph.D. and a minimum of two years of postdoctoral experience in biochemistry, cell biology, pharmacology, or a related field is required. Must have demonstrated experience in GPCR biology through a proven track record of assay development, practical application of techniques, and peer-reviewed publications. Other qualifications include an in-depth knowledge of the GPCR literature as well as excellent communication and team skills. Visit **website: http://www.encysive.com** to submit curriculum vitae and to learn more about Encysive Pharmaceuticals.

POSTDOCTORAL POSITION
HARVARD MEDICAL SCHOOL

Positions available to study DNA damage and the molecular biology of aging and neurodegenerative diseases. Projects focus on DNA damage repair and the biology of genes involved in aging, Alzheimer's and Parkinson's disease. For reference see: *Nature* **429:**883, 2004; *J. Biol. Chem.* **280:**36895, 2005; *Hum. Mol.Genet.* **14:**1231, 2005; *Neuron* **33:**677, 2002. Experience in molecular biology and genetically engineered mouse models is desirable. Send curriculum vitae, a brief description of research experience and names of three references to: **Dr. Bruce Yankner, e-mail: bruce.yankner@childrens.harvard.edu; fax: 617-730-1953.**

POSITIONS OPEN

The Division of Nutrition and Metabolic Diseases at the **University of Texas Southwestern Medical Center, Dallas** seeks to hire a **POSTDOCTORAL FELLOW/RESEARCH SCIENTIST** to study genetically modified mice created to understand how genes involved in triglyceride and phospholipids synthesis lead to fat loss and diabetes. The laboratory provides opportunity to conduct research in genetics, molecular biology, cell imaging, microarray, and proteomics.

Interested candidates with Ph.D. degree or equivalent and experience in molecular biology and lipid biochemistry should submit curriculum vitae to **e-mails: abhimanyu.garg@utsouthwestern.edu** and **anil.agarwal@utsouthwestern.edu.**

University of Texas Southwestern is an Equal Opportunity, Affirmative Action Employer.

POSTDOCTORAL POSITIONS, Department of Cellular Biology, University of Georgia, Athens. Projects in trypanosomes and apicomplexan parasites on: (1) aquaporins and osmoregulation; (2) calcium signaling and storage; (3) polyphosphate function; and (4) isoprenoid pathway. Experience in molecular biology or biochemistry required. Send curriculum vitae and names of references to: **Roberto Docampo (e-mail: rdocampo@uga.edu),** or **Silvia Moreno (e-mail: smoreno@uga.edu).** For more information visit **websites: http://www.uga.edu/cellbio/docampo.html** or **http://www.uga.edu/cellbio/moreno.html.**

POSTDOCTORAL POSITION

Postdoctoral position is available to study cell and molecular and biology of complications of diabetes mellitus and kidney development. Potential candidates must have documented experience of one or more years in cell and molecular biology techniques. Please send curriculum vitae with three references to: **Yashpal S. Kanwar, M.D., Ph.D., Northwestern University Medical School, 303 E. Chicago Avenue, Chicago, IL 60611, U.S.A. E-mail: y-kanwar@northwestern.edu.**

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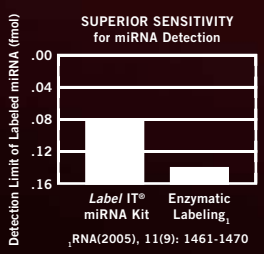
① MICRORNA LABELING

Proven performance with Species Specific Arrays from CombiMatrix, NCode™ Arrays from Invitrogen, *mir*Vana™ Arrays from Ambion, and miRMAX™ Arrays from Rutgers University.



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